

## **DOCTORAL THESIS**

**Identifying the behavioural and neural mechanisms that may explain why late chronotype individuals are at increased risk of developing major depressive disorder**

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Identifying the behavioural and neural mechanisms that may  
explain why late chronotype individuals are at increased risk  
of developing Major Depressive Disorder.

by

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A thesis submitted in partial fulfilment of the requirements for the  
degree of PhD

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# LAY SUMMARY

Depression is a serious and debilitating disorder that is estimated to affect 322 million people worldwide. It is now known that ‘night owls’ (people that prefer to go to bed late and wake up late) are more likely to develop depression compared to ‘morning larks’ and intermediate individuals. However, the mechanisms underlying this increased risk are not fully understood. Here, healthy individuals completed computer-based tasks, an online experiment and underwent magnetic resonance imaging (MRI) in order to explore how night owls respond to emotional information compared to morning larks. It was found that night owls process emotions in a more negative way; for example, they remembered more negative than positive words, were better at identifying sad rather than happy faces and were less responsive to rewards. When the function of the brain was investigated, night owls showed altered activity within regions of the brain responsible for processing and regulating emotions, and showed altered connectivity within a key brain network when at rest. However, the structure of the brain and the levels of chemical messengers within the brain did not depend on whether you were a night owl or morning lark. These findings suggest that differences in the way that night owls interpret emotions may help to explain their increased vulnerability to developing depression (although this does not mean that they *will* develop depression). Importantly, these findings should help us develop strategies to prevent these people from developing depression as well as promoting positive mental health.

# ABSTRACT

Introduction: Major Depressive Disorder is estimated to affect 322 million people worldwide. Increasing evidence suggests that late chronotype individuals (or ‘night owls’) are at increased risk of developing depression. However, the underlying cognitive and neural mechanisms that confer risk are not fully understood.

Methods: Here, healthy individuals without a current or previous diagnosis of depression, family history of depression or sleep disorder were recruited and their chronotype estimated. Participants completed lab-based behavioural tasks, an online facial expression recognition task and underwent magnetic resonance imaging (MRI) in order to examine cognitive and neurophysiological functioning in relation to chronotype.

Results: The results indicated that late chronotypes display negative emotion processing biases compared to early chronotypes, including self-referent memory and recognition of personality trait words and facial expression recognition. In particular, late chronotypes showed biases towards negative stimuli and/or away from positive stimuli and also displayed a blunted response to reward during a risk-taking task. Unexpectedly, late chronotypes recalled fewer words compared to early chronotypes suggesting general memory impairments that may also be relevant to risk for depression. The neural substrates of the negative biases were further illustrated using MRI. Later chronotype was associated with enhanced amygdala reactivity to fearful vs. happy facial expressions and reduced functional connectivity between amygdala and dorsal anterior cingulate cortex. Moreover, later chronotypes displayed reduced resting-state connectivity within key nodes of the default mode network. These findings highlight the putative neural mechanisms underlying altered emotion processing/regulation and self-critical thoughts in this population. In contrast, these neurocognitive differences were not accompanied

by negative attentional biases, structural differences in brain regions associated with emotion processing (e.g. hippocampus) or changes in key neurometabolites (GABA, glutamate, NAA).

Discussion: The current findings appear consistent with theories emphasising negative cognitive biases in the aetiology and maintenance of depression and may, in part, explain the increased vulnerability for depression in late chronotype individuals. These neurocognitive biases are thought to represent trait vulnerability markers for depression and have important theoretical and clinical implications for the prevention of the disorder, and for promoting psychological well-being in late chronotypes.

“The early bird catcheth the worm” – John Ray.

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"In order to succeed, we must first believe that we can" - Nikos Kazantzakis.

# DECLARATION

Several chapters included in this thesis have been previously published:

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# **CHAPTER ONE**

## **Literature Review**

### **1.1. General Introduction**

Depression (Major Depressive Disorder) is a common mental illness that presents with symptoms including low mood, anhedonia (loss of interest or pleasure in hobbies and activities), feelings of guilt, low self-worth, low energy, as well as altered sleeping patterns, appetite and libido, and disturbances in motivation, cognition, memory and attention (WHO, 2012). The World Health Organisation (WHO) estimates 322 million people worldwide are affected by depression and it is one of the leading causes of disability. The global annual prevalence of depression is estimated at 4.4% (4.5% in the United Kingdom) and is more prevalent in women (5.1%) than men (3.6%) (WHO, 2017). Overall, depression is estimated to affect 10% - 15% of the population over their lifetime (Bromet et al., 2011; Lim et al., 2018). Each year, it is estimated that almost 1 million people commit suicide due to depression with an additional 20 million people attempting suicide (WHO, 2012), and suicide is the leading cause of death for males aged 20-49 years in England and Wales (Office for national statistics, 2015). It is therefore a major health concern that has a large impact on the patient themselves, their family as well as health services.

Although major depression is recognised as a severe mental illness that is different from feelings of general 'sadness', defining this disorder has proved to be difficult. As reviewed by Maj (2011), three approaches have been considered that aim to differentiate depression. First, the contextual approach argues that depression is different to normal sadness when there is either a disproportionate reaction to a life event (e.g. severe depressive symptoms, prolonged

duration of symptoms or enhanced functional impairment) or depressive symptoms that are unrelated to a life event. Second, the qualitative approach suggests that the boundary between sadness and depression is subjective/qualitative and that the oversimplification of diagnostic criteria often disregards this difference. Third, the pragmatic approach assumes that depressive states exist on a continuum and that a threshold for diagnosis is determined in order to identify individuals that need clinical attention and pharmacological treatment for severe symptoms (Maj, 2011). The pragmatic approach is the most widely accepted approach and accordingly, the DSM criteria determines that an individual must report several persistent symptoms of depression for at least two weeks to meet the diagnostic criteria for the disorder. However, there are problems with all three approaches, including; defining what a disproportionate response to an adverse life event involves, characterising the qualitative differences in symptoms, and defining what the clinical threshold for depression should be. Therefore, defining depression is an ongoing challenge.

Another problem with the definition of depression is that the diagnosis encompasses a wide range of symptoms. The diagnostic category of 'Major Depressive Disorder' was originally created by the DSM-III in 1980 and although this diagnosis has been widely used in medicine and research, it is essentially a very broad term for a highly heterogeneous disorder. In fact, Shorter (2014) argues that the success of the popular antidepressant Prozac can be directly related to the introduction of the 'major depression' category because it simplified what the drug could be used to treat and a general scientific mechanism of action was advertised i.e. blocking serotonin reuptake in the brain (Shorter, 2014). However, it is well known that patients with major depression have considerable variation in symptoms, course, genetics and treatment response. For example, only five of nine symptoms including one of two core symptoms are needed in order for major depression to be diagnosed, meaning that patients with the same disorder may only have one symptom in common (van Loo, de Jonge, Romeijn, Kessler, &

Schoevers, 2012). Similarly, opposite symptoms of hypersomnia, hyposomnia, weight gain and weight loss all fall into the same category of major depression. Several attempts have therefore been made to create homogenous subtypes of depression. For example, melancholic depression is a subtype characterised by a specific set of symptoms including melancholic features e.g. anhedonia and a lack of mood reactivity. Similarly, psychotic depression includes symptoms of psychosis such as delusions and hallucinations. Subtypes can also be characterised in relation to the onset of the disorder (e.g. seasonal affective disorder, postpartum depression, early-onset and later life depression) its course (single episode, recurrent and chronic depression) and severity (van Loo, de Jonge, Romeijn, Kessler, & Schoevers, 2012). For example, in a large prospective population-based study of patients with a first episode of depression; approximately 15% did not recover (do not have a year free of depression), 35% remitted but had 1 or more future episodes and the remaining 50% had no future episodes after 23 years (Eaton et al., 2008). In another study, it was reported that 25% of individuals experience their first depressive episode before the age of 18 and 50% before the age of 25 (Sorenson, Rutter, & Aneshensel, 1991) whilst Al-Harbi (2012) reported that approximately 30% of patients with depression did not respond to any type of treatment (Al-Harbi, 2012). Therefore, it is important to note that depression is a difficult disorder to define and that there is a considerable amount of variability in the symptoms, course and outcome of depressive episodes between patients. This should be taken into consideration because a large number of studies of depression use the DSM criteria of MDD and it may therefore be difficult to compare participants and findings between studies. In recent years, there has been a drive to study depression in terms of symptoms, severity and duration instead. Nonetheless, major depression is a major health concern in the UK and across the world and new treatments and prevention strategies for the disorder are a top priority for clinical and psychological research.

Current theories of the aetiology and maintenance of depression emphasise altered cognitive, biological and neural processes. For example, depressed patients have been reported to display negative biases in attending to, interpreting and recalling emotional information as well as global cognitive deficits (see section 1.3) which support cognitive theories of depression. Biological theories of depression are based on evidence for altered stress reactivity and impaired functioning of the hypothalamic-pituitary-adrenal (HPA) axis in depressed patients (see section 1.4). Recent advances in neuroimaging techniques (particularly MRI) have also contributed to our understanding of the neural mechanisms that underlie these impairments in depression. For example, depression has been associated with altered structure and function of brain regions involved with emotion processing and regulation which may relate to the negative cognitive biases observed behaviourally (see Chapter 4). However, these theories and their potential mechanisms do not emphasise a causal model for the onset of depression i.e. it is unclear whether these cognitive/biological/neural abnormalities represent a ‘state’ or ‘trait’ marker for depression. A state factor suggests the abnormalities are an indication of current depression whereas a trait factor suggests they are present before the onset of depression which could represent a vulnerability marker for the disorder. This distinction is vital in order to understand the disease course of depression and to develop prevention strategies for the disorder.

Research has also identified key risk factors that make certain (healthy) individuals more vulnerable to depression; including family history of depression, childhood adversity and high neuroticism. These factors are thought to interact with a trigger such as a major life event (e.g. divorce, death of a close relative) to increase vulnerability for depression. Previous research has investigated some of the cognitive, biological and neural mechanisms involved with these risk factors. However, in recent years, a large corpus of evidence from large cohort studies have also identified late chronotype (or ‘evening preference’) as a separate risk factor



for depression (see section 1.6.6.). To date, exploration of the cognitive and neural mechanisms that underlie this association has been limited and so the present research sought to identify vulnerability markers for depression in relation to chronotype using behavioural and neuroimaging techniques. Identification of cognitive biases and neural mechanisms in healthy, late chronotype individuals could represent trait markers for depression which has important implications for the prevention of the disorder.

Therefore, in this introductory chapter, cognitive and biological theories of depression will be outlined and the literature surrounding negative processing biases and impaired stress reactivity associated with depression will be discussed. This chapter will evaluate the existing literature based on behavioural findings whereas Chapter 4 will discuss neural findings relating to the neuroimaging literature. Following this, a review of the literature identifying healthy populations that are at increased risk for depression will be reported. The last section will focus on chronotype and the evidence for late chronotype as a risk factor for depression which will form the basis for the rest of this thesis. Since this association is specific to major depression, the current review (and the rest of the thesis) will be focused primarily on research relevant to depression whilst avoiding disorders that are often reported as comorbid e.g. anxiety. Finally, the objectives and chosen methodology of the current thesis investigation will be summarised.

## 1.2. An integrated theory of depression

Current research suggests there is unlikely to be a single cause of depression. Instead, a combination of factors are known to contribute towards the development of depression; including genetic, biological, environmental and psychological factors, and generally a ‘stressor’ such as major life event e.g. bereavement, divorce or trauma. This is termed the

diathesis-stress model which proposes that depression is the result of an interaction between vulnerability to the disorder and environmental stress including a perceived loss of vital investment. Recently, Beck and Bredemeier (2016) have provided an integrated model of depression in which they suggest there are six levels which contribute to our understanding of the disorder; genetics, neuroanatomy, personality, neurochemicals, an evolutionary framework and a clinical framework (Beck & Bredemeier, 2016). They propose that the development of depression starts with risk factors such as genetic risk and early experiences/trauma. This predisposition to depression contributes to the development of information processing biases (e.g. in attention and memory) and biological reactivity to stress (e.g. altered HPA axis functioning) which are reflected in structural and functional alterations in the brain. Over time, this can lead to negative cognitive patterns known as the 'cognitive triad' and depressogenic beliefs. The cognitive psychology of depression was first described in Beck's cognitive theory which outlines three concepts (Beck, 1989). First, the cognitive triad posits that depressed patients display negative cognitive patterns regarding themselves, their environment/experiences and their future which result in the symptoms of depression including negative affect, lack of motivation, increased dependency and physical symptoms. Second, depression is maintained by dysfunctional 'schemas' or ideas that inform the individual about what to expect from a situation, which are activated and cause the individual to lose control over their thought processes. Third, the validity of negative beliefs are maintained through faulty information processing despite contradictory evidence. These beliefs are then thought to further contribute towards negative processing biases and stress reactivity allowing the depressed state to be maintained. The following sections will describe the behavioural evidence for Beck's cognitive theory of depression including; the range of negative processing biases and abnormal reactivity to stress associated with depression.

### 1.3. Information processing biases

As discussed earlier, Beck's cognitive theory of depression suggests that individuals that process information negatively and experience increased biological reactivity to stress (resulting from vulnerabilities such as genetics and childhood trauma) are more likely to develop depressogenic thoughts and beliefs. These depressogenic thoughts and beliefs in turn allow negative cognitive biases to persist and the depressed state to be maintained. In support of this, it is well established that depressed patients display a range of negative biases in attention, recognition and memory of emotional information and self-referential information as well as differences in risk-taking and reward-seeking behaviours. The details of these findings are discussed below.

#### 1.3.1. Attentional biases to emotional stimuli

Previous work focusing on attentional biases to emotional stimuli have reported differences between depressed patients and healthy controls. A commonly used paradigm to measure attentional bias is the dot-probe. In this task participants are required to view pairs of neutral and emotional stimuli (e.g. words, faces, pictures) presented simultaneously and followed immediately by a probe replacing one of the two stimuli. Participants are required to indicate which side of the computer screen the probe is presented on as quickly and accurately as possible. Trials where the probe replaces the emotional stimulus are referred to as congruent trials whereas incongruent trials occur when the probe replaces the neutral stimulus. Faster reaction times in response to congruent vs. incongruent trials are interpreted as increased vigilance or an attentional bias towards the emotional stimulus. A review of 29 studies using the dot-probe task indicated a significant moderate attentional bias (Cohen's  $d$  effect size = 0.52) towards negative, mood-congruent information in depressed individuals compared to

normal controls (Peckham, McHugh, & Otto, 2010). The authors reported a similar negative attentional bias effect (although smaller effect size) in depressed patients in studies that used a modified Stroop task where participants are asked to name the font colour of emotional words (Peckham, McHugh, & Otto, 2010). Attentional biases towards negative emotions have been displayed in depressed patients (Gotlib, Krasnoperova, Yue, & Joormann, 2004), healthy participants that have undergone depressed-mood induction (Bradley, Mogg, & Lee, 1997) and remitted patients (Joormann, Talbot, & Gotlib, 2007).

However, past studies of depression using the dot-probe task have also described conflicting findings. It is now largely agreed that presentation timing of the stimulus is important for revealing attentional biases as there is stronger evidence for mood congruent biases in depression when stimuli are presented for longer durations i.e. 500 ms or more (De Raedt & Koster, 2010) although there are inconsistencies (Hill & Dutton, 1989). For example, Joormann and Gotlib (2007) reported that depressed and remitted patients selectively attended to sad facial expressions when stimuli were presented for 1000 ms (Joormann & Gotlib, 2007) and Mogg and colleagues (1995) reported an attentional bias towards negative words exclusively in the longer presentation time condition (1000 ms) but not for the shorter subliminal condition (14 ms) in depressed patients (Mogg, Bradley, & Williams, 1995b). Moreover, a stronger effect to attend to socially threatening words was found in a longer (500 ms) versus shorter (50 ms) presentation condition in depressed patients (Mathews, Ridgeway, & Williamson, 1996). Indeed, use of the shorter presentation time is proposed to capture the initial allocation of attention and the longer presentation time is thought to capture maintenance of attention. Therefore, findings of negative attentional biases in depression are interpreted as a reduced ability to disengage from the emotional stimulus, especially when the information is self-relevant (Karin Mogg & Bradley, 2005). Whereas more robust findings for subliminal attentional biases have been reported in anxious patients (Mathews et al., 1996) or more

severely depressed patients (Trapp, Kalzendorf, Baum, Hajak, & Lautenbacher, 2018) suggesting a reduced ability to allocate attentional resources to the stimulus initially.

Taken together, previous evidence suggests depressed patients display negative attentional biases towards emotional information and specifically, increased time to disengage from negative emotional stimuli compared to healthy controls. Inconsistencies between studies are likely to reflect differences in the depressed population studied e.g. anxiety comorbidity and depression severity.

### 1.3.2. Recognition and recall biases of emotional stimuli

Whilst findings of attentional biases in depression are inconsistent, memory biases are largely considered a more reliable marker of the disorder (Marchetti et al., 2018). As well as being associated with over-generalised autobiographical memory (Kohler et al., 2015), depressed individuals have been shown to preferentially remember information that is mood-congruent i.e. negative or unpleasant memories that are consistent with depressed mood (Bradley, Mogg, & Williams, 1995). This negative bias has been observed during both implicit and explicit memory paradigms. For example, implicit memory tasks include word stem completion tasks where participants have to fill in the missing letters of a word stem from memory, or lexical decision tasks where participants are asked to identify if emotional stimuli are words or non-words. Performance on these tasks allow previously primed words to be remembered without deliberate recall of the experience. By contrast, explicit memory tasks include free recall of emotional information or recognition tasks where participants are asked to identify if stimuli are 'old' (presented before) or 'new' (novel words). Performance on these tasks represents recall of information using strategic processes. As suggested by Graf and Mandler (1984), implicit memory represents activation and integration of mental

representations whereas explicit memory represents elaboration of mental representations and these are distinct processes (Graf & Mandler, 1984).

In a meta-analysis of explicit memory biases in depression, Matt and colleagues (1992) reported that depressed individuals exhibited preferential recall of negative information, individuals with dysphoria (a state of unease) did not recall positive or negative information preferentially and healthy controls showed preferential recall for positive information (Matt, Vázquez, & Campbell, 1992) suggesting a state-dependent effect on recall. Additionally, recall biases in depression have been specifically related to depressive negative information (e.g. words relating to sadness and low mood) rather than other types of negative information e.g. danger-related. This further demonstrates biases towards mood-congruent memory, similar to attentional biases reported in depression. It is thought that explicitly recalled information requires greater depth and elaboration of processing which are more likely to influence attitudes, interpretations of the environment and mood (Ingram, 1984).

There is also considerable evidence that implicit memory biases exist in depression and a recent meta-analysis reported that depressed mood is reliably associated with preferential recall of negative words (mean effect size is small-medium, Cohen's  $d = 0.31$ ) (Gaddy & Ingram, 2014). It is also recognised that these implicit memory biases are better revealed when the processing demands of the encoding and recall tasks are congruent. This relates to the transfer appropriate processing framework which suggests that information retention depends on both perceptual and conceptual processes (Morris, Bransford, & Franks, 1977). In a perceptual processing paradigm, information is processed quickly and shallowly whereas in a conceptual processing paradigm the information is semantically processed. For example, depressed patients were reported to recall significantly more negative words when both encoding (dot-probe task) and recall (word completion task) tasks were perceptually processed (Taylor & John, 2004). Similarly, another study reported that depressed patients demonstrated

more priming of negative words than controls when both encoding (studying word-list) and recall (free association task) were conceptually processed (Watkins, Vache, Verney, Muller, & Mathews, 1996). Although some studies have reported implicit memory biases for tasks that have not used matching encoding and recall tasks as well (Ruiz-caballero & Gonzilez, 1994).

Taken together, both explicit and implicit memory biases have been robustly identified in depression. Explicit mood-congruent memories are thought to be more accessible in depression and therefore contribute to the maintenance of the disorder. Similarly, implicit biases in memory are thought to further mediate negative cognitions through unconscious and automatic processing.

### 1.3.3. Biased facial expression recognition

In addition to the studies described above that investigated emotional verbal biases, a range of experiments have examined negative biases towards facial expressions. Using facial expressions as stimuli is arguably a more reliable and ecologically valid method of testing emotion recognition as faces are key to visual communication and interpretation of social cues. There are two common paradigms used to test facial expression recognition. In the first paradigm, participants are instructed to categorise the emotion of a particular expression e.g. happy, sad, fearful, angry, disgusted, surprised (Ekman & Friesen, 1971). In general, depressed patients perform worse on this task than healthy controls for a range of emotions; for example, Flanagan and colleagues (2011) reported patients with depression had reduced accuracy to recognise happy and fearful facial expressions and patients with postpartum depression were less able to recognise disgusted and angry expressions (Flanagan, White, & Carter, 2011). Additionally, depressed patients show difficulties in verbally labelling the emotion of facial expressions (Feinberg, Rifkin, Schaffer, & Walker, 1986). In fact, Tremeau and colleagues

(2005) reported that depressed patients were less able to imitate emotional expressions, reflect a particular emotion expression themselves and had less spontaneous expression of emotion using speech, smiling and gestures assessed via interviews (Tremeau et al., 2005). These findings are consistent with evidence of emotional blunting observed in severely depressed patients.

The second paradigm used to test facial expression recognition is the morphed face paradigm. This paradigm uses faces that are manipulated from 0% (i.e. neutral expression) to 100% emotion (e.g. very sad) usually in increments of 10% where participants are asked to accurately categorise the emotion. This allows responses to emotional, neutral and ambiguous faces to be recorded. As well as difficulties in recognising emotional facial expressions overall, depression is consistently associated with a processing bias towards negative facial stimuli and away from positive facial stimuli using this paradigm. Previous research has described a specific increase in perception for negative, sad facial expressions (Bouhuys, Geerts, & Gordijn, 1999) whilst other studies have described a specific decrease in perception for positive, happy facial expressions (Sloan, Strauss, & Wisner, 2001) or both a tendency for depressed patients to interpret neutral faces as sad and happy faces as neutral (Gur et al., 1992). One study that provided insight into these inconsistencies identified that depressed patients displayed significantly impaired discrimination accuracy for sad facial expressions only when stimuli were presented for relatively brief periods (100 ms) whereas patients also tended to label ambiguous happy faces (50% intensity) as neutral only when faces were presented for more sustained periods (2000 ms) (Surguladze et al., 2004). This suggests that depression is characterised both by general deficits in recognising emotional facial expressions and emotion-specific biases in facial expressions which can be revealed by changing the emotional intensity and presentation time of the stimuli. Surguladze and colleagues (2004) also reported that the observed facial recognition biases were greater in participants with more severe symptoms of



depression, and patients with higher doses of antidepressant medication had reduced response accuracy to ambiguous sad faces compared to patients with lower doses (Surguladze et al., 2004) suggesting another possible explanation for inconsistencies between studies.

Taken together, biases in processing and recognising facial expressions have been consistently identified as markers of depression, particularly biases towards negative facial expressions. This impairment is relevant to the negative interpretations of the environment and social interactions often observed in depressed patients.

#### 1.3.4. Negative biases to self-referential stimuli

As outlined earlier in Beck's cognitive model, depression is often characterised by negative schemas relating to the 'self' which when activated by negative life events allow the depressed state to be maintained. For example, depressed individuals that hold negative self-referential schemas are more likely to perceive incoming information as personal criticisms or flaws. This is evidenced by research showing that depressed patients are more likely to identify negative versus positive adjectives as self-referential (Davis, 1979). Many tasks investigating negative biases in attention, recognition and recall in depression have been adapted to directly probe these self-critical schemas. For example, Ji and colleagues (2017) designed an attention task that first asked participants in the 'self-referential' condition to categorise positive and negative adjectives regarding themselves whilst participants in the 'other-referential' condition categorised the words in reference to a neutral celebrity. Then participants underwent a dot-probe task using the same words. More severely depressed patients showed significantly reduced attention to positive words in the self-referential condition compared to the other-referential condition (Ji, Grafton, & MacLeod, 2017). In another attention task, depressed patients showed greater interference for self-descriptive adjectives during a modified Stroop

task than for non-self-descriptive adjectives (Segal, Gemar, Truchon, Guirguis, & Horowitz, 1995).

Similarly, emotion recognition and recall tasks have also been adapted to reflect self-referential stimuli. For example, in one emotion categorisation paradigm, participants are presented with personality trait words and are told to 'imagine that they have overheard someone else describing them using this word'. This allows negative biases towards self-referential personality trait words to be identified. Similarly, recall biases towards self-referential personality trait words can be tested using this adaptation. Compared to healthy controls, depressed patients have been shown to freely recall more negative than positive personality trait words, but only in self-referent conditions (Bradley & Mathews, 1983). In a recent meta-analysis, an overall significant effect on mood-congruent memory for negative information was found on implicit tasks as well (e.g. a fragment completion task where participants are asked to complete the word) where self-reference was a significant moderator across studies that used these tasks (Gaddy & Ingram, 2014). Fewer studies have investigated negative processing biases to facial expressions in relation to the self. However, in one study, Bouhuys and colleagues (1995) reported that participants that had undergone depressed mood induction perceived ambiguous faces to be more 'rejecting', and non-ambiguous faces to be less 'inviting' than before the mood induction (Bouhuys, Bloem, & Groothuis, 1995a). This suggests that depressed mood alters the perception of social signals that are self-directed.

Taken together, many adapted paradigms investigating attention, recall and information processing have revealed specific negative biases in self-referential processing. This supports Beck's cognitive theory that self-referent schemas and self-critical thoughts are a key symptom and mechanism underlying the maintenance of depression.

### 1.3.5. Altered risk-taking and reward-seeking behaviours

As well as the core symptom of persistent low mood in depression, anhedonia and cognitive dysfunction are also cardinal symptoms of the disorder. Anhedonia refers to reduced interest or pleasure in everyday activities, whereas cognitive dysfunction refers to impairments in attention, learning, memory, information processing, problem solving, risk taking and motor function. In relation to these symptoms, depression has been associated with impairments of the reward system where individuals display altered sensitivity and learning to reward and punishment (Eshel & Roiser, 2010). Deficits in the reward system can be assessed during risk-based tasks that require decision making. These tasks allow individuals to make a decision based on the opportunity to gain a reward whilst trying to avoid potential punishment. A range of tasks have been used to assess risk-taking and reward-seeking behaviours including the Balloon Analogue Risk Task (BART), delayed discounting task, IOWA gambling task and many more that often use point systems or financial incentives as rewards. For example, the BART task provides a measure of risk-taking by asking participants to make successive ‘pumps’ on each trial to inflate a balloon. By increasing the number of pumps, the participant can win more points/money but this also increases the chance that the balloon will burst and the reward earned on that trial will be lost (punishment). Using this task, Hevey and colleagues (2017) reported that depressed individuals entered a significantly lower number of pumps overall and this was correlated with higher levels of depressive symptoms. Specifically, depressed individuals also adjusted (reduced) the number of pumps significantly more after experiencing a loss (burst balloon) compared to healthy controls suggesting increased sensitivity to punishment (Hevey, Thomas, Laureano-Schelten, Looney, & Booth, 2017).

As reviewed by Eshel and Roiser (2010), depression has been associated with both punishment avoidance and a reduced capacity to focus on rewards (Eshel & Roiser, 2010). In support of the hyposensitivity to reward hypothesis, Henriques and Davidson (2000) reported

that depressed patients did not adjust their patterns of response for trials where recalling a specific word was rewarded with money, compared to normal controls that did (Henriques & Davidson, 2000). Similarly, Pizzagalli found that depressed patients and individuals with high BDI scores were not able to develop a preference for responding to a particular target that was three times more likely to be rewarded compared to healthy controls (Pizzagalli, Jahn, & O'Shea, 2005). Moreover, impaired responsiveness on this reward task was found to predict higher symptoms of anhedonia after one month. Interestingly, a meta-analysis of six studies using probabilistic reward tasks showed that depression was associated with an impaired ability to modulate their behaviour in response to reward rather than impaired learning on the task (Huys, Pizzagalli, Bogdan, & Dayan, 2013). Finally, Pulcu and colleagues (2014) reported impairments on a delayed discounting task in depressed patients where participants preferred to take smaller rewards with a shorter delay time than healthy participants (Pulcu et al., 2014). These findings suggest a blunting response to reinforcement in depression although could also indicate impaired future-thinking i.e. negative perceptions of the future.

In addition to hyposensitivity to reward, there is also evidence that depression is associated with an impaired response to punishment. For example, Steffens et al (2001) administered a timed Trail Making Test to participants which involved drawing a trail between consecutive alternating numbers and letters whilst performance errors were immediately fed back to the participant. Depressed patients made more subsequent errors on the task after an initial error was identified compared to healthy controls (Steffens, Wagner, Levy, Horn, & Krishnan, 2001). Similarly, depressed patients were found to significantly deteriorate in performance on the Tower of London Planning Task after they had made an error on a trial, despite solving each problem equally well as healthy controls (Beats, Sahakian, & Levy, 1996). This suggests that depressed patients have maladaptive responses to perceived failure which

has been interpreted as either hypersensitivity to punishment or an inability to use negative feedback to improve performance.

Neuroimaging studies suggest that reward-related decision making is supported by a distributed network of brain regions modulated by monoamine systems including dopamine. This includes the nucleus accumbens, caudate, putamen, thalamus, orbitofrontal cortex (OFC), insular, anterior cingulate cortex (ACC), posterior cingulate cortex, inferior parietal lobule and prefrontal cortex (Liu, Hairston, Schrier, & Fan, 2011). Different regions of this network are involved in processing different aspects of reward. For example, Liu and colleagues (2011) suggested that the ventral striatum and medial OFC are implicated in representing the value of the reward and detecting positive reward valence whereas the lateral OFC, anterior insula, ACC and amygdala are implicated in detecting negative reward valence. The anterior insula and ACC are also thought to be involved with anticipating reward/loss during risky decisions whilst fronto-parietal regions, in particular the OFC, integrate this information to form optimal decisions (Liu et al., 2011). In relation to this, fMRI studies of depression have reported hypoactivity of the striatum, caudate and putamen during reward-related tasks and blunted activity within the ventral striatum (including nucleus accumbens, ventral caudate and ventral putamen) has been specifically related to symptoms of anhedonia (Keedwell, Andrew, Williams, Brammer, & Phillips, 2005; Wacker, Dillon, & Pizzagalli, 2009). Deficits within reward circuitry therefore suggest that depressed patients are unable to use affective information to guide their decision-making behaviour effectively.

Taken together, depression is associated with impaired responses to negative feedback. This is evidenced by studies showing increased sensitivity to punishment and/or a blunted response to reward and is consistent with symptoms of anhedonia often experienced in depression. Neuroimaging studies also suggest impaired detection and integration of positive/negative outcomes within reward circuitry may be responsible for impaired reward

sensitivity and altered risk taking behaviour. This cognitive dysfunction is likely to decrease the likelihood of being exposed to rewards as well as distorting information from the environment to confirm these biases; further exacerbating depressive symptoms.

## 1.4. Stress reactivity

As well as negative processing biases, abnormal biological reactivity to stress is thought to be involved in the pathway to depression. Indeed, dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis has been consistently reported in severe mood disorders such as depression and bipolar disorder (Young, 2004). The HPA axis is a major neuroendocrine system comprised of the hypothalamus, the pituitary gland and adrenal cortex in a complex network of feedback loops that receives neuronal input and releases a number of hormones. Briefly, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the paraventricular nucleus of the hypothalamus which cause adrenocorticotrophic hormone (ACTH) to be released from the anterior lobe of the pituitary gland. This hormone causes cortisol to be secreted from the adrenal cortex. Cortisol is a key glucocorticoid that has a range of effects on physiological processes including; the immune system, mood and energy storage, mediated via glucocorticoid receptors. Regulation of the HPA axis is tightly controlled via neuronal inputs from the amygdala, hippocampus and midbrain, and by cortisol itself (via negative feedback loops to the hypothalamus and pituitary). The HPA axis therefore controls reactivity to stress and regulates a number of physiological processes (Young, 2004). Cortisol is often used as an indicator of HPA axis function (although other markers such as ACTH are also used). Cortisol displays a circadian rhythm in which a significant increase (50% -75%) is observed within the first 30 minutes after waking followed by a decline to baseline after approximately 1 hour (termed the Cortisol Awakening Response (CAR)). Cortisol is therefore usually measured in saliva samples

with strict reference to time of waking to provide dynamic measurement of HPA activity (Pruessner et al., 1997) whilst hair cortisol has generally been used to assess long-term fluctuations in stress.

There is considerable evidence that cortisol plays a central role in the pathogenesis of depression, however the nature of its role still remains unclear. For example, there is evidence for disrupted cortisol rhythms in roughly 50% of depressed patients (Sachar et al., 1973), resistance of the HPA axis to respond to glucocorticoid feedback in roughly 50% of depressed patients (Carroll, Martin, & Davies, 1968), an increase in the CAR in healthy individuals at-risk for depression (Portella, Harmer, Flint, Cowen, & Goodwin, 2005), prolonged exposure to corticosteroids causing the development of depression (Lewis & Smith, 1983) and higher mean morning cortisol levels predicting onset of depression (Harris et al., 2000). Therefore, it is thought that elevated levels of cortisol (reflecting impaired reactivity to stress) are related to the onset of depression through breakdown of the feedback mechanisms within the HPA axis (Young, 2004). It has also been shown that hypercortisolemia can lead to neuronal atrophy (particularly of the hippocampus, mediated by glutamate) (Sapolsky, 2000) and impairments in learning and memory (Young, Sahakian, Robbins, & Cowen, 1999) associated with depression.

However, there are inconsistencies relating to cortisol hyperactivity in depression. For example, Stetler and Miller (2005) found blunted CAR in a group of moderately depressed women and this was not related to waking time or social interaction compared to healthy controls (Stetler & Miller, 2005). Similarly, Strickland and colleagues (2002) reported a reduced CAR in depressed patients and no changes in cortisol in vulnerable individuals (Strickland et al., 2002). In fact, in a meta-analysis of 361 studies investigating HPA axis function in depression, Stetler and Miller (2011) reported an overall increase in cortisol in depressed individuals but this effect was reduced by half (from  $d = 0.60$  to  $0.33$ ) when only studies using rigorous cortisol sampling methods were used (Stetler & Miller, 2011) suggesting

different methodology may be a possible explanation for the inconsistencies observed across studies. In addition, Belanoff and colleagues (2001) reported increased levels of afternoon cortisol in patients with psychotic major depression compared to patients with non-psychomotor major depression and healthy controls (Belanoff, Kalehzan, Sund, Fleming Ficek, & Schatzberg, 2001), and a meta-analysis of 20 studies showed older adults suffering from depression displayed higher dysregulation of the HPA axis than younger depressed adults (Belvederi Murri et al., 2014). This suggests that depression sub-type and age may also impact on cortisol levels in depression.

Taken together, HPA axis dysfunction and biological reactivity to stress is widely accepted as a marker for depression. However, inconsistencies surrounding whether cortisol is increased or decreased in depression remain unclear and could be related to the heterogeneous nature of the disorder (e.g. sub-types of depression), participant characteristics (e.g. age) or methodological issues involved with cortisol sampling.

## 1.5. Vulnerability to depression

Overall, there is substantial evidence for abnormalities across a range of cognitive and biological processes in depression. However, it remains unclear whether these abnormalities represent a ‘state’, ‘trait’ or ‘scar’ marker of depression. A state factor suggests the abnormalities are an indication of current depression whereas a trait factor suggests they are present before the onset of depression, and could, therefore, reflect a vulnerability marker for the disorder. A scar effect is considered to be a long-lasting abnormality in cognitive and neurobiological processes that arises as a consequence of previous depression, and a number of neural abnormalities have been reported in remitted depressed patients (Anderson et al., 2011; Bhagwagar, Cowen, Goodwin, & Harmer, 2004; Merens, Booij, & Van Der Does, 2008) similar



to currently depressed individuals. The distinction between these three categories of state, trait and scar effects is vital in order to understand the disease course of depression and, importantly, to develop prevention strategies. The focus of the current investigations was to identify trait markers of depression in relation to chronotype and participants recruited to the following studies are healthy (no current or previous diagnosis of depression). Therefore, the following sections identify other high-risk populations where negative biases have been identified that may represent trait markers for depression. This is then followed by a more detailed review of late chronotype as a risk factor for depression which will be the focus of this thesis.

#### 1.5.1. Genetic predisposition

Based on genetic research such as twin and family studies, it has been established that risk for depression has a genetic component. In a recent meta-analysis, it was estimated that the heritability of major depression is between 31-42% (Sullivan, Neale, & Kendler, 2000) meaning that the proportion of variation in depression that can be explained by genetics is around 37% in the general population. A large number of candidate gene and genome-wide association studies (GWAS) have tried to identify specific gene variants associated with major depression. However, these studies have largely produced inconclusive or un-replicated findings and there is still not a clear consensus about which specific genes contribute towards predisposition. This is partly due to studies that are underpowered but also due to the heterogeneous nature of depression which makes it difficult to determine which measures of depression (e.g. length of episodes, number of episodes, number of symptoms, type of symptoms, onset of depression) are associated with specific genes (Flint & Kendler, 2014). It is also very likely that vulnerability to depression is polygenic.

On the other hand, investigations of gene-environment interactions in depression have yielded more promising findings. For example, possessing one or two copies of the short allele of the serotonin transporter-linked polymorphic region (5-HTTLPR) of the SLC6A4 gene has been linked to increased prevalence of depression and suicide following a stressful life event (Caspi et al., 2003). Similarly, a variant of the FKBP5 gene (which is involved with modulating glucocorticoid receptors) has been shown to predict the onset of severe depression (Zimmermann et al., 2011), as well as depressive symptoms and prognosis of depression (Binder et al., 2004; Lekman et al., 2008), in combination with an adverse life event. Genetic polymorphisms in IL6 and IL1 $\beta$  genes (associated with pro-inflammatory molecules) have been shown to moderate the effect between depressive symptoms and exposure to interpersonal stress but not other stressors such as financial, work and health-related difficulties (Tartter, Hammen, Bower, Brennan, & Cole, 2015). Numerous other genes have also been implicated in gene-environment interactions including CRHR1, NRC31 and BDNF although issues surrounding the type and timing of environmental exposure still exist in these studies (Klengel & Binder, 2013). These findings indicate a number of genes that increase vulnerability to depression in combination with life stressors, however it also highlights the complex nature of depression i.e. genetics alone cannot predict the onset of depression and in many cases, individuals that are both genetically predisposed to depression and have experienced a major life event do not necessarily develop the disorder. It is therefore important to understand different risk factors for depression.

### 1.5.2. Early experiences

One of the most established risk factors for depression (and indeed a number of other psychiatric conditions) is experience of childhood adversity. For example, a retrospective study by Parker and colleagues (1995) reported that participants with a diagnosis of depression (176

adults out of a sample of 3684) were significantly more likely to report low parental care during childhood than healthy controls (Parker, Hadzi-Pavlovic, Greenwald, & Weissman, 1995). Depression has also been associated with childhood physical or sexual abuse, marital discord and experience of family violence (Bifulco, Brown, Moran, Ball, & Campbell, 1998; Harkness & Monroe, 2002; Young, Abelson, Curtis, & Nesse, 1997), and it is estimated that experience of childhood adversity in clinical samples of depressed patients is between 8% - 83% and 23% - 68% in community samples (Lenze, Xiong, & Sheline, 2008). In a 10 year prospective study, Klein and Kotov (2016) found that participants that experienced childhood adversity at baseline were more likely to report higher levels of chronic depression (Klein & Kotov, 2016) and there is also accumulating evidence that childhood adversity is associated with an earlier onset of depression (Young et al., 1997) and a poorer recovery outcome from depression (Zlotnick, Ryan, Miller, & Keitner, 1995). Strikingly, a large cohort study (n = 17,337) also reported that the risk of suicide was increased by 2- to – 5 fold in participants that had experienced a range of adverse childhood experiences compared to participants that had not (Dube et al., 2001).

The proposed mechanism that explains the association between experience of childhood adversity and depression is that the individual is sensitised to later stress which contributes to the risk of depression. For example, depressed adolescents that reported a history of childhood abuse or neglect were more likely to report a lower level of independent stressful life events prior to their first episode of depression compared to adolescents that had not reported childhood adversity (Harkness, Bruce, & Lumley, 2006). Additionally, there is evidence that individuals that have experienced childhood adversity (by virtue of parental loss or separation) were more likely to become depressed specifically following lower levels of interpersonal loss (e.g. relationship breakups and death) rather than other types of loss (e.g. financial problems) (Slavich, Monroe, & Gotlib, 2011). In comparison to genetic predisposition for depression, severe negative experiences in childhood may be an independent risk factor for depression.

However, genetic and environmental risk factors are not mutually exclusive and there is increasing evidence that they influence one another. For example, childhood trauma was found to increase the risk of psychiatric disease e.g. PTSD, by DNA methylation of the FKBP5 gene leading to stress-dependent gene transcription causing dysregulation of the stress hormone and immune system (Klengel et al., 2013). Equally, stressful life events that are known to increase risk for depression e.g. marital quality, have been shown to have a heritable component (Kendler & Baker, 2007). Together, these findings suggest childhood adversity is a potent risk factor for depression that sensitises individuals to subsequent stress.

### 1.5.3. Neuroticism

As well as genetic and environmental risk factors, high neuroticism has been described as a major predictor for depression (Kendler, Kuhn, & Prescott, 2004). Neuroticism is one of the big-five personality traits and refers to an individual's predisposition to experience negative emotions e.g. anger, fear, frustration (Eysenck., 1964). Highly neurotic individuals therefore tend to experience more mood swings. This trait has been shown to be relatively stable over an individual's lifespan and has a heritability of approximately 43% (Wray, Birley, Sullivan, Visscher, & Martin, 2007). In a longitudinal study of 1733 female twins, neuroticism was strongly related to life time prevalence of depression and predicted onset of major depression 15 months later (Kendler, Neale, Kessler, Heath, & Eaves, 1993) and a study of a large sample of Swedish twins replicated these findings in a 25-year follow up (Kendler, Gatz, Gardner, & Pedersen, 2006). High neuroticism in premorbid patients has also been shown to be significantly correlated with episode duration in depression as well as dysfunctional attitudes and over-general autobiographical memories independent of depression severity (Scott, Williams, Brittlebank, & Ferrier, 1995). This finding has also been replicated in non-Western populations; for example, neuroticism was found to be associated with lifetime prevalence of

depression and comorbid disorders in a large sample of Chinese female participants. Moreover, depressed patients were more likely to report a younger age of onset, more episodes of depression and longer duration of episodes if they had higher neuroticism scores (Xia et al., 2011). As well as a trait marker for depression, experiencing an episode of depression has been shown to elevate scores of neuroticism in remitted patients suggesting a scar effect as well (Reich, Noyes, Hirschfeld, Coryell, & O'Gorman, 1987).

The relationship between neuroticism and depression has been shown to exist largely due to shared genetics (about 0.46) (Kendler, Gatz, et al., 2006). However a large proportion remains that can be explained due to environmental influences. This is consistent with Beck's theory of depression and 'cognitive vulnerability hypothesis' in which maladaptive thinking patterns are thought to increase the risk for depression when interacting with adverse life events.

#### 1.5.4. Cognitive vulnerability

In addition to individuals that display high neuroticism personality trait, vulnerability to depression is thought to be related to cognitive styles. Again, this is reported in Beck's 'cognitive vulnerability hypothesis' where an individual develops maladaptive beliefs about oneself, their environment and the future (a negative cognitive triad) as well as negative 'schemas' that are consistent with these depressogenic beliefs. In response to Beck's theory, the Hopelessness theory was developed by Abramson and colleagues (1989) as a second cognitive theory for depression. According to this theory, negative events experienced by the individual are attributed to internal, stable and global circumstances that are beyond their control. This specific inference style puts the individual at risk of depression following stressful life events because it diminishes their self-worth and autonomy (Abramson, Alloy, & Metalsky, 1989). Another cognitive style which is thought to predict depression and suicidal thoughts is

rumination. Rumination is characterised by an inflexible and perseverative cognitive style which places a repeating focus on the cause, meaning and consequences of low mood (Nolen-Hoeksema, 2000). This cognitive style is thought to interfere with problem-solving and mood regulation which are known to be associated with depression (Watkins & Baracaia, 2002).

In support of negative cognitive styles as a risk factor for depression, Alloy and colleagues (2006) reported that healthy individuals with a high-risk cognitive style (as assessed using the Cognitive Style Questionnaire and the Dysfunctional Attitudes Scale) were 3.5 - 6.8 times more likely to develop depression over 2.5 years compared to low-risk cognitive style individuals and were more likely to have anxiety comorbidity too (Alloy et al., 2006). Similarly, cognitive high-risk participants were more likely to experience more episodes of depression, more severe episodes and higher chronicity of depression than low-risk participants in a prospective design (Iacoviello, Alloy, Abramson, Whitehouse, & Hogan, 2006). Finally, rumination (brooding and reflection) was found to be a significant predictor of suicidal ideation after one-year follow-up in a large community sample of adults (Miranda & Nolen-Hoeksema, 2007).

Taken together, the key risk factors that make an individual more vulnerable to depression are genetic predisposition, experience of childhood adversity, high neuroticism personality trait and a maladaptive cognitive style. Each risk factor is thought to contribute to information processing biases and impaired biological reactivity to stress which, in combination with a trigger, can lead to depressogenic thoughts and beliefs. However, traditional theories of depression have largely excluded the influence of chronotype in depression which has now also been recognised as a significant predictor of depression. Therefore, the next sections will review the literature surrounding chronotype and describe the evidence for late chronotype as a risk factor for depression.

## 1.6. What is chronotype?

Chronotype, or morningness-eveningness refers to individual differences in diurnal preference (Horne & Ostberg, 1976b), i.e. a measure of preferred bed time, get-up time, tiredness in the morning and peak performance. Chronotype is measured along a scale although it is commonly organised into three broad circadian groups: early chronotypes (colloquially referred to as ‘morning larks’), that rise early and reach their peak of cognitive and physical performance early in the day (Schmidt, Collette, Cajochen, & Peigneux, 2007; Schmidt et al., 2012). By contrast, late chronotypes (or ‘night owls’) prefer later bed and wake times and would typically schedule meetings, exercise sessions etc. later in the day ( i.e. “synchronised” to an individual’s optimal time of day according to their circadian profile). Whereas intermediate chronotypes (also referred to as neither types) fall between the two. Chronotype is a normally distributed trait present in the general population and is considered a relatively stable trait although patterns across the lifespan are recognised (see section 1.6.4.).

### 1.6.1. The biological clock

An individual’s chronotype is determined by the human circadian clock. It is generally accepted that there are three different ‘clocks’ that humans are entrained to; the biological clock (genetic component), the solar clock (light and temperature cues) and the social clock (work and social constraints) (Roenneberg, Wirz-Justice, & Mellow, 2003). In humans, the central pacemaker is the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus, which controls the biological clock. The SCN displays rhythms in metabolism, electrical activity and gene expression and receives input from environmental signals (zeitgebers) to synchronise the circadian clock to the 24 hour day. It also synchronises circadian oscillators in other brain

regions, cells and peripheral organs in a hierarchical system via neural outputs and the production of hormones (Gillette & Tischkau, 1999).

The biological clock is responsible for generating the internal day via complex molecular mechanisms thought to be based upon the regulation of clock genes and their products. The core clock genes in humans include: period genes *Per1*, *Per2* and *Per3*, cryptochrome genes *Cry1* and *Cry2* and their expression is regulated at the transcription level via proteins *BMAL1* and *CLOCK* (Wulff, Porcheret, Cussans, & Foster, 2009). The neurones in the SCN, where these clock genes are expressed, are cellular oscillators that coordinate the circadian clock. The clock genes are active at the early part of the night where their proteins begin to accumulate. However, once these proteins gain access to the nucleus of the cell, they suppress their own genes and protein synthesis stops. After a lag time, the existing proteins are broken down, *BMAL1* and *CLOCK* proteins stimulate transcription and the clock genes can begin protein synthesis again in a molecular feedback loop. The process takes about 24 hours and is self-sustaining (Hastings, 1998).

One of the functions of the circadian rhythms generated by the SCN is to regulate the sleep/wake cycle via the production of hormones such as melatonin and cortisol. The SCN neurones decrease in firing rate late in the day which stimulates sympathetic activity and triggers the onset of melatonin release from the pineal gland (Moore, 2007). Levels of melatonin begin to rise in the evening (about 2 to 3 hours before sleep onset) which triggers drowsiness and a dip in core body temperature, levels remain high over night, and then drop in the early hours of the morning to trigger wakefulness (Hardeland et al., 2011). The SCN also controls release of cortisol from the adrenal gland which peaks approximately 30 minutes after waking causing an awakening response (Cortisol Awakening Response (CAR)) via the formation of blood glucose. In addition to circadian processes, the other regulatory system that influences



the sleep/wake cycle is homeostatic sleep pressure where the ‘pressure’ to sleep accumulates after a number of hours awake.

There is increasing evidence that the biological clock influences an individual’s chronotype. In fact, a study of twins estimated that 37% of diurnal preference can be accounted for by genetic influences (Watson, Buchwald, & Harden, 2013). A recent genome-wide study identified 12 new genetic loci that are associated with morningness-eveningness (Lane et al., 2016). For example, a mutation in the *Per3* gene has been associated with chronotype where the long allele version of the repeat region is linked with extreme morning preference, whereas the short allele version is linked with extreme evening preference (Archer et al., 2003). However, this population study was based on a UK sample and observations of this mutation in a population sample from Brazil were the opposite (Pereira et al., 2005) suggesting that there are cultural differences in the clock genes too. A number of mutations in clock genes have also been associated with circadian pathology. For example, a single mutation on chromosome 2 of the *Per2* gene has been linked to Familial Advanced Sleep Phase Syndrome (FASPS) – a disorder that severely advances a person’s chronotype (Fu, Jones, Toh, Virshup, & Ptacek, 2001). Similarly, a mutation in the *Cry1* gene has recently been linked to Delayed Sleep Phase Disorder (DSPD); responsible for a significant (often 2 hour) delay in circadian rhythm (Patke et al., 2017).

### 1.6.2. Zeitgebers

As well as genetic influences, there are a number of different zeitgebers (environmental cues) that entrain the circadian clock to the 24-hour day and influence chronotype. The most significant zeitgeber is light exposure which allows the internal biological clock to be synchronised to the light/dark cycle via the photoreceptor system within the eye. Specialised

photosensitive retinal ganglion cells (pRGCs) receive light input and release glutamate and pituitary adenylate cyclase activating polypeptide (PACAP) at their synapses with the SCN. This in turn activates an intracellular signalling cascade that activates the transcriptional activator CREB (cAMP-response binding element binding protein). This transcription factor then upregulates the clock genes *Per1*, *Per2* and *Dec1* which results in the prolonged suppression of *CLOCK* and *BMAL1* transcription proteins and modulation of the internal molecular clock to the light cycle (Challet & Pevet, 2003). This in turn alters the pattern of activity in the SCN and its tightly controlled functions, including the melatonin cycle. This mechanism controls both diurnal and seasonal rhythms; progression from autumn to winter causes the melatonin signal to lengthen and equally, progression from winter to spring causes the signal to shorten (Hastings, 1998). For example, Johnsen and colleagues (2013) reported that a sample of Norwegian participants were slightly earlier chronotypes (by 8 minutes) when assessed during the summer compared to the winter (Johnsen, Wynn, Allebrandt, & Bratlid, 2013).

The significance of the light/dark cycle to the circadian clock has been identified through comparing individuals living in rural and populated areas. In one study, the chronotype of Germans that lived in villages and towns with less than 300,000 inhabitants moved proportionally with the progression of dawn. Whereas, Germans that lived in cities had chronotypes that correlated much weaker with daylight hours and their chronotype was on average more evening-orientated (Roenneberg & Merrow, 2007). This is explained by the fact that inhabitants of large cities are often exposed to less natural day light and therefore their circadian clock is not entrained to the light/dark cycle as precisely. The strength of the zeitgeber has also been shown to be important (Vetter et al., 2011). Vetter and colleagues reported that sleep timing and timing of activity on free days advanced significantly with the time of dawn (over a 5 week period from January to February) when office workers were exposed to more

natural yellow light during their working day, compared to office workers that were exposed to artificial 'blue' light that synchronised their circadian clocks to their office hours (Vetter, Juda, Lang, Wojtysiak, & Roenneberg, 2011). Artificial light is thought to alter circadian rhythms by inhibiting melatonin production (Gooley et al., 2011). This also highlights the negative effect of artificial light on sleeping patterns and health which is becoming increasingly problematic with the growing use of technology.

Without the influence of environmental cues such as the light-dark cycle, the endogenous human circadian rhythm oscillates within a period that is slightly longer than the 24 hour day. This is referred to as 'free-running' and has been demonstrated in mice exposed to 24 hour darkness and in blind people with a lack of physiological light response (Lewy & Newsome, 1983). However, the circadian rhythm can also be entrained to the 24-hour cycle in the absence of light cues. For example, Sack and colleagues (2000) reported that administering an oral dose of melatonin an hour before preferred bed time caused six blind participants to entrain to a 24 hour cycle (Sack, Brandes, Kendall, & Lewy, 2000). There are a number of non-photic cues including; the sleep-wake cycle, physical activity/exercise, temperature, meal times, social contact, melatonin and stimulants (Caldelas, Chimal-Monroy, Martinez-Gomez, & Hudson, 2005; Mistlberger & Skene, 2005). These cues significantly affect circadian timing and chronotype but are not as potent as light cues.

### 1.6.3. Measuring chronotype

Chronotype can be measured in a number of ways including core body temperature, peak melatonin secretion, cortisol awakening levels, actigraphy and a number of self-report questionnaires. For example, the average minimum temperature for early chronotypes was found to occur at 03.50 hours, at 05.02 hours for neither chronotypes and at 06.01 hours for late

chronotypes (Baehr, Revelle, & Eastman, 2000). Acrophase (peak time) of cortisol has also been shown to occur on average 55 minutes earlier in early chronotypes, and has a higher amplitude than late chronotypes (Bailey & Heitkemper, 2001). Finally, the average Dim Light Melatonin Onset (DLMO) was shown to be 21:48 for early chronotypes and 24:36 for late chronotypes (Lack, Bailey, Lovato, & Wright, 2009). The most common self-report method of measuring chronotype is using the Morningness-Eveningness Questionnaire developed by Horne and Östberg (Horne & Ostberg, 1976a). The questionnaire consists of 19 multiple choice questions probing when individuals prefer to go to bed and wake up, perform mental and physical tasks, their subjective alertness throughout the day and their level of morning affect (how tired they feel in the morning). Chronotype is scored on a scale from 16 to 86, where lower scores represent evening preference and higher scores represent morning preference. Importantly, the MEQ has been validated by correlating scores with biological measures of chronotype. For example, MEQ scores have been shown to strongly correlate with average minimum temperature ( $r = -0.52$ ) (Baehr et al., 2000) and DLMO ( $\text{Beta} = -0.03$ ,  $p < 0.01$ ) (Burgess & Fogg, 2008). The MEQ has also been revised into a shortened version (5 questions), the reduced MEQ (rMEQ), which is often used in chronotype studies in the interest of time (Adan & Almirall, 1991b). The rMEQ shows good test-retest correlations with the MEQ ( $r = 0.768$ ) and between chronotype groups ( $r = 0.524$ ) (Carciofo, Du, Song, Qi, & Zhang, 2012), it has been validated against measures of actigraphy (Vincenzo, José, Monica, & Marco, 2006) and is considered a reliable measure of chronotype (Di Milia, Adan, Natale, & Randler, 2013).

Other self-report measures of chronotype also exist which assess different aspects of circadian timing. For example, the Munich Chronotype Questionnaire (MCTQ) focuses more on sleep timings and phases (Roenneberg et al., 2003). The questionnaire probes sleep times on both work days and free days since most individuals, except extreme early chronotypes, accumulate sleep debt over work days and extreme early chronotypes may accumulate sleep

debt over free days if adapting to late social schedules. Chronotype is calculated based on an individual's mid-sleep i.e. the midpoint between going to sleep and waking up and this can be corrected for sleep deficit. This measure of chronotype has been validated using the MEQ as a comparison where mid-sleep on free days (MSF) was highly negatively correlated ( $r = -0.73$ ) with sleep preference scores from the MEQ. This measure (MSF) has also been correlated with DLMO (Martin & Eastman, 2002) and average daily cortisol minimums (Roenneberg et al., 2004). One limitation of the MCTQ scale is that it requires participants not to use an alarm clock on free days which is difficult to enforce, and it is quite long. The Composite Scale of Morningness (CSM) is a third method of measuring chronotype that was developed by extracting questions from other questionnaires including the MEQ (Smith, Reilly, & Midkiff, 1989). The CSM shows good internal consistency; for example, an alpha value of 0.87 was shown in sample of French nursing students (Caci, Deschaux, Adan, & Natale, 2009). However, a limitation of this questionnaire compared to the MEQ and MCTQ is that it has not yet been validated against physiological markers of chronotype such as body temperature, melatonin and cortisol levels.

#### 1.6.4. Individual differences

Although chronotype is considered a relatively stable trait, a number of different patterns across an individual's lifespan have been recognised. These findings have been identified in large cross-sectional studies of healthy undergraduate students and adults. For example, studies that have assessed chronotype in particular age groups have reported children to be more morning orientated (Zimmermann, 2016), leading to a dramatic swing towards eveningness in adolescents (Carskadon, Labyak, Acebo, & Seifer, 1999) followed by a gradual shift towards morningness in older adults (Randler, Freyth-Weber, Rahafar, Florez Jurado, & Kriegs, 2016). Similar findings were also reported in a large sample ( $n = 25,000$ ) of healthy

German individuals where, on average adolescents reach their peak ‘lateness’ around the age of 20 years (Roenneberg et al., 2004). Differences in chronotype can also be found between genders. In the same sample, Roenneberg and colleagues reported that women reached their maximum lateness at 19.5 years, compared to 20.9 years for men, and have on average an earlier chronotype for most of their adult life (16 – 50 years) (Roenneberg et al., 2004). This corresponds with the general tendency for females to develop earlier than males and this sex difference is prominent during female reproductive years but disappears at the average age of menopause (50 years). This has been corroborated by most large scale studies including a meta-analysis by Randler (2007) that showed a small but significant effect of gender on morningness (Randler, 2007).

Individual differences in sleep quality and length are also related to chronotype. For example, Bavarsad and colleagues reported a significant negative correlation between MEQ scores and sleep quality (as assessed by the Pittsburgh Sleep Quality Index) in female students such that individuals with a late chronotype had poorer sleep quality (Bavarsad, Azimi, Moradbeigi, & Latifi, 2015). Indeed, early chronotypes have been found to have higher sleep efficiency (Lehnkering & Siegmund, 2007), longer sleep duration (Kitamura et al., 2010) and more regular sleep schedules than late chronotypes (Ong, Huang, Kuo, & Manber, 2007). Chronotype has also been investigated across a number of studies in relation to academic achievement and personality. For example, a meta-analysis of college students reported that late chronotype was weakly associated with better cognitive ability (assessed via measures of intelligence) but poorer academic achievement (Grade Point Average) (Preckel, Lipnevich, Schneider, & Roberts, 2011). In a large sample ( $n = 1343$ ) of American university students, late chronotype was found to predict poorer extrinsic motivation, less agreeableness and less conscientiousness (Onder, Besoluk, Iskender, Masal, & Demirhan, 2014). Similarly, later chronotype has been associated with higher scores of novelty- and sensation-seeking alongside

reduced scores of persistence compared to early chronotypes (Antúnez, Navarro, & Adan, 2014). Late chronotype has also been related to openness, extraversion and neuroticism although these findings are less consistent (Christoph Randler, Schredl, & Göritz, 2017).

#### 1.6.5. Synchrony effects

A body of research has been dedicated to uncovering ‘synchrony effects’ associated with chronotype. The synchrony effect refers to the improvement in performance on a number of cognitive tasks when testing occurs during an individual’s optimal time of day i.e. late chronotypes perform better in the evening whereas early chronotypes perform better in the morning. For example, May and Hasher (1998) reported that individuals performed better on an inhibitory control garden-path task (where participants had to remember alternative endings to a sentence when cued) when they were tested at their optimal time of day dependent on their chronotype (May & Hasher, 1998). Similar findings have been reported across a variety of tasks including; visual and verbal memory tasks (Intons-Peterson, Rocchi, West, McLellan, & Hackney, 1999), working memory tasks (Rowe, Hasher, & Turcotte, 2009), executive function tasks (Hahn et al., 2012) and problem-solving tasks (May, 1999), although not all studies have shown these effects including a study of long-term memory (Barbosa & Albuquerque, 2008). Previous evidence also suggests that synchrony effects may be dependent on age as many studies have reported this finding to be more prominent in older adults (Intons-Peterson et al., 1999; Rowe et al., 2009) and one study found that older adults with an intermediate chronotype performed at their peak at midday for inhibitory processing, executive function, long-term memory and forgetting compared to younger adults with an intermediate chronotype that did not show any synchrony effects (May & Hasher, 2017).

There is also evidence for asynchrony effects i.e. improved performance during non-optimal periods (NOP), for tasks that involved implicit/unconscious processing. For example, Rowe and colleagues (2006) reported that participants had better implicit memory for distractors during a word priming task when they were tested at a non-optimal time-of-day compared to an optimal time-of-day (Rowe, Valderrama, Hasher, & Lenartowicz, 2006). Similarly, participants that were assigned to a condition where they completed an artificial grammar learning task based on implicit memory at a non-optimal time-of-day (based on self-report) performed better than participants assigned to the optimal time-of-day condition (Delpouve, Schmitz, & Peigneux, 2014). This suggests that performance on implicit tasks may be improved at off-peak times-of-day because attentional control is reduced allowing automatic processes to be enhanced. Whereas tasks that require effortful attentional processing may be better performed at optimal times of the day.

#### 1.6.6. Late chronotype as a risk factor for depression

A growing body of research suggests that late chronotype is associated with negative health conditions including obesity, metabolic disorders and cardiovascular disease (Fabbian et al., 2016). In addition, increasing evidence indicates an association between late chronotype and psychological health – particularly depression. For example, Hidalgo and colleagues (2009) observed a 5-fold increase in the likelihood of reporting moderate to severe depressive symptoms (as measured using the Montgomery-Asberg Depression Rating Scale) in 200 healthy individuals with a late chronotype as compared to early or intermediate chronotypes (Hidalgo et al., 2009). Similarly, Levandovski and colleagues (2011) reported higher Beck Depression Inventory scores in late chronotypes as compared to early and intermediate types in a large population sample free of sleep disorder and psychoactive drug use (Levandovski et al., 2011). Merikanto and colleagues (2013, 2015) reported that late chronotypes were significantly



more likely to report depressed mood, anhedonia, a diagnosis of depression or use of prescribed antidepressant medication (Merikanto et al., 2015; Merikanto et al., 2013) and this association has also been shown to be more prominent in younger adults (Kim et al., 2008). Importantly, these findings have been replicated in large-scale studies across cultures including; 10,503 Finnish adults (Merikanto et al., 2015), 1944 Dutch adults and 641 young Korean adults (Park et al., 2015).

In many of these large cohort studies, a number of covariates are included in the regression models to minimise the effects of confounding variables. For example, Antypa and colleagues (2016) reported that late chronotype was a significant predictor of depression even after adjusting for sociodemographic details (including age, gender, education, employment, having a partner and living with children), somatic health factors (including BMI, chronic disease, smoking and alcohol consumption) and sleep-related factors (including insomnia and sleep duration) (Antypa, Vogelzangs, Meesters, Schoevers, & Penninx, 2016). In particular, smoking, alcohol consumption and drug use (e.g. cannabis use) may be potential confounding variables underlying this association because there is some evidence that excessive alcohol consumption and illicit drug use during adolescence increases the risk of self-reported depression in adulthood (Brook, Brook, Zhang, Cohen, & Whiteman, 2002), and late chronotypes are twice as likely to smoke cigarettes, are more likely to drink alcohol and use cannabis compared to early and intermediate chronotypes (Patterson, Malone, Lozano, Grandner, & Hanlon, 2016; Urban, Magyarodi, & Rigo, 2011). Similarly, there is evidence that late chronotypes suffer from poorer sleep quality, insufficient sleep, excessive daytime sleepiness and problems initiating sleep compared to intermediate and early chronotypes (Yun, Ahn, Jeong, Joo, & Choi, 2015) suggesting that sleep disturbances may also be a confounding factor underlying the association between late chronotype and depression. Indeed, Wittmann and colleagues (2010) reported a significant association between late chronotype and depressive

symptoms in a sample of 500 healthy participants and this relationship was partially mediated by smoking and alcohol use (although the best explanation for the variance was age) (Wittmann, Paulus, & Roenneberg, 2010). Similarly, poor sleep quality has been reported to partially mediate the relationship between late chronotype and depression (Horne, Watts, & Norbury, 2018). However, both these studies (Horne et al., 2018; Wittmann et al., 2010) reported that smoking, alcohol use, drug use and sleep quality do not fully mediate the relationship and a number of large studies that control for these health-related factors (e.g. Antypa et al., 2016; Kitamura et al., 2010; Merikanto et al., 2013; Merikanto et al 2015) suggest that an independent link between later chronotype and depression exists.

Much of the evidence for a link between late chronotype and depression has been shown to be largely specific to major depression. For example, Antypa and colleagues (2016) reported that chronotype was not related to dysthymia (persistent mild depression) or anxiety disorders (including panic disorder, agoraphobia, social anxiety disorder and generalised anxiety disorder) but was significantly related to major depression (as measured using the Composite International Diagnostic Interview) (Antypa et al., 2016). However, other studies have provided evidence that late chronotype may be a risk factor for affective disorders in general. For example, two studies reported that patients with bipolar disorder and anxiety disorder were more likely to be late chronotypes (Fares et al., 2015; Lemoine, Zawieja, & Ohayon, 2013). However, these findings are equivocal (Au and Reece., 2017) and other studies have suggested that later chronotype is more directly associated with depressive symptoms in bipolar disorder (rather than mania) and increased comorbidity (including anxiety) in depression (Melo, Abreu, Linhares Neto, de Bruin, & de Bruin, 2017; Reid et al., 2012). As mentioned in section 1.1, major depression is a broad term that encompasses multiple symptoms and so previous studies of patients with a diagnosis of major depression provide more of a general picture of what late chronotype constitutes risk for. Therefore investigating how late chronotype relates to different

aspects of depression (e.g. symptom profiles, course, duration and severity) provides more detailed analysis which may be more relevant. To date, there have been some studies that have investigated how late chronotype relates to these factors. For example, later chronotype has been associated with worse psychological distress, increased symptom severity, higher suicidality, more impairments in work and everyday activities, worse mood in the morning and non-remission in patients with major depression (Chan et al., 2014; Fares et al., 2015; Gaspar-Barba et al., 2009). Equally, chronotype was not associated with duration of illness or number of hospitalisations (Chan et al., 2014). Taken together, previous findings suggest that late chronotype is a risk factor for depression and in particular, severe symptoms of low mood. Late chronotype may also be relevant to other affective disorders such as bipolar disorder and anxiety but most studies have identified a specific link with major depression as this diagnosis includes the core symptom of persistent low mood. Although these cross-sectional findings clearly demonstrate an important link between late chronotype and depression, they do not speak to the causal nature of the relationship.

More recently, longitudinal studies have deconstructed the association between late chronotype and depression further. In one study of 255 adolescents (11-19 year olds) from the community, chronotype was found to be a significant predictor of future onset of a depressive episode (odds ratio = 0.92) such that individuals with a late chronotype were more likely to have a future depressive episode and report significantly higher depressive symptom scores one year later even after controlling for prior depression and pubertal status using logistic regression (Haraden, Mullin, & Hankin, 2017). Similarly, Van Den Berg and colleagues (2018) reported late chronotype was a significant predictor of elevated depressive symptoms at 1 year follow up in a sample of Dutch students (Van den Berg, Kivela, & Antypa, 2018). In another longitudinal study of 32,470 female nurses, definite early chronotypes were significantly less likely (adjusted hazard ratio = 0.88) than intermediate chronotypes to report a diagnosis of

depression 4 years later even when a number of variables were taken into consideration (menopausal state, marital status, living situation, income, retirement status, smoking status, physical activity, alcohol consumption, BMI and sleep duration). Definite late chronotypes were not significantly more likely to develop depression compared to intermediate chronotypes although overall, there was a significant linear trend of increasing depression risk across chronotype categories (Vetter et al., 2018). Limitations of this study were that chronotype was determined based on one question from the MEQ and only definite early and late chronotypes were compared against an 'intermediate' chronotype group (defined as individuals reporting slightly morning, neither and slightly evening chronotypes). Diagnosis of depression was also assessed via self-report. Taken together, recent longitudinal studies extend previous observational data such that late chronotype is a risk factor for future diagnosis of depression independent of confounding variables such as age, gender, drug use and sleep quality, whereas early chronotype may be a protective factor. However, only very few well-designed, longitudinal studies have investigated the directionality of this relationship and so these findings need to be replicated using large scale longitudinal studies that follow up individuals over a longer period of time (> 4 years).

Despite many studies reporting a significant relationship between late chronotype and depression, the effect sizes between studies vary from small to large. For example, a meta-analysis of 36 correlational studies of late chronotype and depression calculated effect sizes according to Fisher's Z score which ranged from -0.04 (Fares et al, 2015a) to -0.51 (Fares et al, 2015b) (Au & Reece, 2017). Overall, the pooled effect size for these studies was -0.20 which according to Cohen's criteria is a small effect (0.2 is small effect, 0.5 is a medium effect and 0.8 is a large effect) (Cohen, 1988). Au and Reece (2017) also reported no evidence of publication bias but that larger effect sizes were present in studies where the Composite Scale of Morningness (CSM) was used to measure chronotype instead of the MEQ. In relation to

longitudinal studies, effect sizes tend to be similarly small although as noted above, these studies are limited and more longitudinal studies are needed in order to establish how strong a risk factor late chronotype is for the development of depression. Nonetheless, evidence suggests there is a small but significant effect of later chronotype on depressive symptoms in clinical and non-clinical populations. This is not unexpected given that there are many genetic, environmental and social factors that influence the development and maintenance of depression, as discussed in section 1.5. Indeed, the relationship between chronotype and depression has been shown to exist partly due to shared genetics: about 0.21 genetic correlation where the genetic covariance between chronotype and depression accounts for approximately 59.1% of the phenotypic correlation (Toomey, Panizzon, Kremen, Franz, & Lyons, 2015) meaning a significant proportion can therefore be accounted for by environmental influences.

One theory to explain why later chronotype is associated with depression is that these individuals experience increased ‘social jet-lag’ i.e. a misalignment between the internal body clock and external demands (e.g. going to work at 9am each morning). In relation to depression, previous evidence has shown that patients are more likely to be late chronotypes (Antypa et al., 2016) and even small shifts in the timing of sleep have an effect on mood. For example, one treatment of depression is sleep deprivation. This involves the individual staying awake for the full night, or from 1am, and the full next day until the evening when they can sleep again. This treatment has been shown to produce a rapid (within 24- 48 hours) antidepressant effect in approximately 50 – 80% of patients, similar to efficacy rates of antidepressant drugs, but also benefits drug-resistant patients (Dallassezia & Benedetti, 2011). Although it is unclear exactly how this treatment works, one theory is that it resets the balance by synchronising the sleep wake cycle to the internal biological clock. According to the ‘internal coincidence hypothesis’ (related to the phase advance hypothesis for depression), depressed patients display circadian rhythms of temperature, hormone release and REM sleep that is phase advanced (earlier)

compared to their sleep-wake cycles and therefore they sleep at the wrong biological time (Wehr, Wirz-Justice, Goodwin, Duncan, & Gillin, 1979). In addition, the S-deficiency model proposes that depression is associated with a lack of sleep-need which normally builds up in the evening before sleep (Borbely & Wirz-Justice, 1982). Therefore, sleep deprivation treatments have an antidepressant effect because the sleep-wake cycle is temporally synchronised to the circadian rhythm and the sleep-need is returned. Usually, the antidepressant effects are lost during the recovery sleep.

In healthy individuals, sleep deprivation treatments usually cause increased sleepiness and irritability rather than a direct effect on mood, however small differences have been reported according to chronotype. For example, Selvi and colleagues (2007) reported a significant increase in depressive symptoms in healthy individuals with an early chronotype and a significant decrease in depressive symptoms in late chronotypes after both total and partial sleep deprivation (Selvi, Gulec, Agargun, & Besiroglu, 2007). This further suggests that misalignment of circadian rhythms has an effect on mood. In contrast to depression however, late chronotype is a trait observed in the healthy population and it is thought that their circadian misalignment is due to social demands and natural light cues that occur at non-optimal times of day according to their internal body clock (i.e. early in the morning when later chronotypes prefer to sleep) which act as stressors and cause low mood. Sleep deprivation treatments are therefore better for treating depression because it targets the biological clock (i.e. sleep-wake cycle and sleep need) which is largely unaffected in healthy individuals with a late chronotype. Other theories proposed to underlie the relationship between late chronotype and depression in healthy individuals are discussed in full in the general discussion.

Taken together, there is evidence from both cross-sectional and longitudinal studies that late chronotype is a risk factor for depression independent of demographic factors, drug and alcohol use and sleep related factors although large prospective studies are still needed. This

relationship relates most strongly to severe symptoms of low mood which is usually encompassed as a diagnosis of major depression. The effect of late chronotype on depression has been shown to be small but significant and understanding the mechanisms underlying this association will contribute towards finding strategies to prevent depression and promote psychological well-being in these individuals.

## 1.7. Thesis investigation

Previous evidence has identified a clear association between chronotype and depression where healthy, never-depressed late chronotypes are at increased risk for developing depression. However, the putative cognitive and neural mechanisms underlying vulnerability to depression in this population are unknown. Cognitive and neural biases in emotional processing and risk-taking have been identified in depressed patients and in other at-risk populations. Therefore, the present work sought to determine if these differences are present in late chronotype individuals that could represent a vulnerability marker for depression. Across the thesis investigation, three samples of healthy individuals were recruited with a range of chronotypes, identified using the Morningness Eveningness Questionnaire (MEQ) (Horne & Ostberg, 1976b). In order to separate out the mechanisms that underlie risk for depression in relation to chronotype, participants were excluded from participation if they reported (via self-report) a diagnosis of depression or history of depression, biological parent with a diagnosis of depression or a diagnosed sleep disorder. Participants were largely undergraduate or postgraduate students at the time of recruitment.

Six cross-sectional experiments were designed including two behavioural studies assessing negative biases in processing emotional information and impaired risk-taking, and four neuroimaging studies. First, a battery of neuropsychological tests was used to assess

negative biases in information processing. This included self-referent categorisation of personality trait words, recognition and recall, facial expression recognition, dot-probe and Balloon Analogue Risk Task (Chapter 2). Second, facial expression recognition was further assessed in a larger, independent sample recruited online (Chapter 3). Finally, four neuroimaging experiments using MRI were conducted where the first experiment examined the neural correlates of implicit facial expression recognition and the remaining three experiments investigated hippocampal structure, resting-state activity and changes in neurometabolites (Chapters 5, 6, 7 and 8) associated with late chronotype. An introduction to MRI and the literature surrounding neural abnormalities in depression will be presented in Chapter 4.

This project was approved by the University of Roehampton Ethics Committee (references: PSYC 15/197, 3YP\_2015\_230, and PSYCH 15/198) and was carried out in accordance with the latest version of the Declaration of Helsinki (World Med, 2013). Details of the methodology and results of each study are reported in the subsequent chapters. The final chapter (Chapter 9) will provide a general discussion of the findings across all six experiments plus limitations and implications for future research.



# CHAPTER TWO

## Negative information processing biases

### 2.1. Introduction

As reviewed in Chapter 1, cognitive theories of depression underline negative information processing biases in the development and maintenance of the disorder (Beck, 1979). Much of the previous literature has focused on five cognitive biases observed in depressed individuals; emotional verbal biases, emotional biases to facial expressions, attentional biases, biases to self-referential information and altered risk-taking/reward-seeking behaviour. For example, depressed patients have been shown to display negative attentional biases towards emotional information and increased time to disengage from negative emotional stimuli (Mogg & Bradley, 2005), preferential recall of negative information (Matt et al., 1992), processing biases towards negative facial expressions and away from positive facial stimuli (Bouhuys et al., 1999; Sloan et al., 2001) and a tendency to interpret negative information as self-referential (Davis, 1979). Depression has also been associated with increased sensitivity to punishment and/or a blunted response to reward resulting in altered risk-taking behaviour (Eshel & Roiser, 2010). Some of these biases have also been reported in remitted depressed individuals (Bhagwagar et al., 2004; Hayward, Goodwin, Cowen, & Harmer, 2005) suggesting they could represent a trait vulnerability factor for the disorder. However, observations in remitted depressed populations could represent a pre-existing cognitive vulnerability to depression or could arise as a consequence of previous depression i.e. a ‘scar’ effect. To address this, it is necessary to examine negative biases in never-depressed individuals at increased risk for developing depression. The following subsections of the introduction will therefore outline

the evidence for negative information processing biases in at-risk individuals, including the existing literature relating to late chronotype.

### 2.1.1. Emotional verbal biases

A number of emotional verbal biases (e.g. categorisation, recognition and recall of words) have been reported in at-risk populations similar to those observed in depressed patients. For example, in an emotional categorisation task, individuals with a high neuroticism score were significantly quicker to categorise disagreeable vs. agreeable self-referent adjectives compared to low neuroticism individuals (Chan, Goodwin, & Harmer, 2007). The authors also reported that highly neurotic individuals had fewer memory intrusions (i.e. fewer mistakes) for agreeable words at subsequent recall (Chan et al., 2007). Similarly, Berdynaj and colleagues (2016) reported that late chronotypes (LC) were significantly slower to recognise agreeable vs. disagreeable personality trait words compared to early-intermediate chronotypes (EICs) and displayed reduced accuracy for recalling the same agreeable personality trait words (Berdynaj et al., 2016).

In relation to healthy individuals with a genetic vulnerability to depression, participants carrying the short 5-HTTLPR allele have been shown to categorise negative self-referential adjectives more easily and this was associated with increased recall of negative adjectives (Dainer-Best, Disner, McGeary, Hamilton, & Beevers, 2018). Similarly, children at high risk of depression (by virtue of having a currently depressed mother) displayed enhanced endorsement of negative self-referent adjectives after a negative mood induction compared with low-risk children (Taylor & Ingram, 1999). Adults that had a biological depressed parent also showed slower response time to recognise positive vs. negative personality trait words (Mannie, Bristow, Harmer, & Cowen, 2007). Additionally, individuals with a negative cognitive style

have been shown to endorse more negative words and fewer positive words as self-referential, were slower to endorse the opposite words as self-referential and recalled significantly fewer positive words on subsequent recall compared to a low risk group (low score on cognitive style questionnaire) (Alloy, Abramson, Murray, Whitehouse, & Hogan, 1997). Finally, in healthy individuals, higher depressive symptoms were associated with faster responses to negative self-referent adjectives, an enhanced memory for self-endorsed negative words and a poorer memory for positive words (Connolly, Abramson, & Alloy, 2016). Together, these findings suggest at-risk populations display negative biases in categorisation, recognition and recall of information, especially when the information is self-referential. It is hypothesised that mood-congruent information is processed more efficiently and encoded more deeply in depression and may be a trait marker for depression as well.

### 2.1.2. Facial expressions

Differences in processing emotional facial expressions have also been reported in at-risk groups. For example, highly neurotic individuals have been shown to have a higher intensity threshold to correctly identify facial expressions as happy compared to a low neuroticism group (Chan et al., 2007). Children that had mothers with a history of depression were significantly more likely to avoid sad (but not happy or angry) faces (Gibb, Benas, Grassia, & McGeary, 2009) and tended to misidentify low-intensity angry facial expressions as sad following a mood induction (Joormann, Gilbert, & Gotlib, 2010) compared to children without a biological depressed mother. Moreover, boys with a familial risk of depression were more likely to categorise ambiguous emotional faces as sad compared to their control peers suggesting an over-sensitivity to sad facial expressions (Lopez-Duran, Kuhlman, George, & Kovacs, 2013). However, in another study of familial risk for depression in healthy adults,

differences in facial expression recognition between groups were not replicated (Mannie, Bristow, et al., 2007). This might be accounted for by the difference in ages between studies (children vs. adults) or that many of the studies in children have also involved negative mood induction. For example, facial processing biases have been shown in healthy participants that have had depressed feelings induced. Bouhuys and colleagues (1995) reported that participants exposed to ‘depressing’ music perceived more ambiguous facial expressions as sad and the most happy facial expressions as less happy (Bouhuys, Bloem, & Groothuis, 1995b). Finally, in relation to chronotype, Berdynaj and colleagues (2016) reported that late chronotypes had enhanced accuracy to identify sad faces compared to early-intermediate chronotypes but no differences were observed for happy facial expressions. Together, there is evidence for negative biases in facial expression recognition paradigms in at-risk populations although studies that have used negative mood inductions may enhance these biases.

### 2.1.3. Attentional bias

As noted in Chapter 1, the evidence for attentional biases in depression is mixed and could rely on the type of stimulus used and the presentation timing. Similarly, attentional biases in at-risk populations have been inconsistent. For example, two studies reported that daughters of depressed mothers selectively attended towards sad facial expressions following a mood induction (Joormann et al., 2007; Kujawa et al., 2011) whereas Gibb and colleagues (2009) reported avoidance of sad faces in a similar sample of children with familial risk for depression but with no mood induction (Gibb et al., 2009). Kujawa and colleagues (2011) also reported a significant sex difference where the attentional bias was displayed in daughters but not sons of depressed mothers (Kujawa et al., 2011). In relation to neuroticism, Chan and colleagues reported no evidence of attentional biases to positive or negative words between high and low

neuroticism groups. There is some debate as to whether faces or words (used as stimuli) are more sensitive to emotional attentional biases, however in a large study of 2257 adolescents, no significant correlation between attentional biases towards happy, angry and fearful expressions and neuroticism personality trait was reported (O’Leary-Barrett et al., 2015) suggesting attentional biases are not present in this at-risk group. Finally, in relation to chronotype, EIC showed a positive attentional bias towards happy facial expressions and a facilitation of attentional resources towards happy congruent trials (probe replaces the happy expression) (Berdynaj et al., 2016). Together, there is evidence for negative attentional biases in some at-risk populations (e.g. familial risk) although inconsistencies between studies may relate to a number of factors such as presentation timing, negative mood induction and sex differences.

#### 2.1.4. Risk-taking/reward-seeking

There are mixed findings relating to altered risk-taking in at-risk groups but there is some evidence that these groups show similar behavioural patterns to depressed patients. For example, older individuals with a high neurotic score have been shown to perform conservatively on the Iowa Gambling Task compared to low neuroticism individuals i.e. they did not learn to choose cards from the ‘good’ decks that provided smaller monetary losses (Denburg et al., 2009). Similarly, young adults with a biological parent with a history of depression performed more conservatively on the Cambridge Gambling task compared to controls i.e. they gambled fewer points on trials where the favourable outcome was more likely (Mannie, Williams, Browning, & Cowen, 2015). Moreover, decreased risk-taking on the same task predicted depressive symptoms and onset of depression 1 year later in a sample of adolescents with a biological depressed parent (Rawal, Collishaw, Thapar, & Rice, 2013).

In relation to chronotype, later chronotype has been associated with self-report measures of impulsivity and novelty-seeking, traits that are associated with risk-taking behaviour (Caci et al., 2005). In particular, Wang and Chartrand showed that late chronotype was related to financially risky behaviours such as gambling and investment rather than health-related risks assessed using scenarios (Wang & Chartrand, 2015). Similarly, Killgore (2007) reported that later chronotype was correlated with greater risk-taking propensity at baseline and after sleep deprivation but was not related to a behavioural measure of risk-taking (adjusted pumps) on the Balloon Analogue Risk Task (BART) (Killgore, 2007). Berdynaj and colleagues (2016) also found no differences in several measures of risk-taking/reward sensitivity on the BART and delayed discounting task (Berdynaj et al., 2016). However, in a recent study by Ingram and colleagues (2016), early chronotypes took significantly more risks (increased adjusted pumps) on the BART task, compared to late chronotypes, and took significantly more risks when they were tested later in the day (Ingram et al., 2016). This suggests that risky decision-making may be affected by synchrony effects and also that there is a difference between self-reported risky behaviours and behavioural measures of risk-taking as early chronotypes reported fewer risky behaviours but took more risks on the BART.

Taken together, there is evidence that altered risk-taking and reward sensitivity may be trait vulnerability markers for depression with findings suggesting a more conservative approach in at-risk individuals. However, comparing findings across different studies is often difficult because of the range of tasks used which reflect measures of decision-making, reward-seeking, impulsivity and sensitivity to financial gains and losses.

### 2.1.5. Study aims

Converging findings suggest depression is associated with altered risk-taking behaviours and negative biases in categorisation, recognition, recall and attention, particularly when the information is self-referent (as discussed in Chapter 1). It is hypothesised that negative (mood-congruent) information that is processed, encoded and recalled to a greater extent can lead to depressogenic thoughts and beliefs associated with the depressed state. It is also suggested that negative bias could be a trait vulnerability marker for depression since high-risk groups (Berdynaj et al., 2016; Chan et al., 2007; Joormann et al., 2007), and remitted patients (Bhagwagar et al., 2004; Hayward et al., 2005) display similar cognitive patterns, and negative biases predict relapse in depressed individuals (Bouhuys et al., 1995b). There is increasing evidence to suggest that late chronotypes represent another population that are vulnerable to depression but the underlying mechanisms of this association are unclear. One method of exploring this relationship is to identify if late chronotypes have biases in emotional processing and risk-taking behaviours that are similar to those identified in depressed individuals.

Therefore, the present study was designed to explore whether negative biases exist in late chronotype participants, similar to those reported in depressed patients, which could reflect a trait vulnerability marker for the disorder. The study also aimed to replicate and extend the findings of Berdynaj et al (2016) to further explore emotional processing biases between chronotype groups. Roehampton university students were recruited with early-intermediate (EIC) and late chronotypes (LC) and without a personal history of depression, family history of depression or diagnosed sleep disorder. Six tasks were chosen to assess different aspects of emotional processing and risk-taking discussed above including emotional categorisation, emotional recall and recognition, facial expression recognition, balloon analogue risk task and dot-probe. The overall hypothesis was that late chronotypes would display affective processing

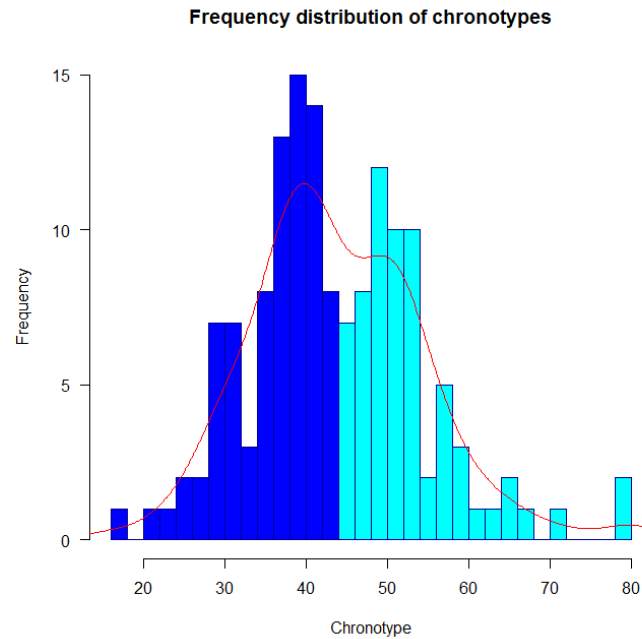
biases towards negative information and/or away from positive information and a conservative risk-taking strategy compared to early-intermediate chronotypes.

## 2.2. Methods

### 2.2.1. Participants

A total of 145 participants completed the emotional categorisation, recall and recognition tasks and 128 completed the facial expression, dot probe and Balloon Analogue Risk Task (BART). These participants were recruited via online advertisement, poster advertisement and personal communication and the majority were Psychology students studying at the University of Roehampton. A proportion of the participants were excluded due to reporting one or more confounding variables including: 1) current diagnosis of depression, 2) family history of depression (first degree biological relative) or 3) sleep disorder. This left a total of 93 participants for the emotional categorisation, recall and recognition tasks (see *Figure 1*) and 82 were included in the facial expression, dot probe and Balloon Analogue Risk Task (BART). The study was approved by the local ethics committee and written informed consent was obtained from all participants before experimental procedures took place.





*Figure 1. Frequency distribution of chronotypes (n = 93). Dark blue shows late chronotypes and light blue shows early-intermediate chronotypes as defined by Horne and Ostberg (1976).*

### 2.2.2. Materials and stimuli

Self-report demographic data including age, gender, number of cigarettes smoked per day and number of units of alcohol consumed per week was collected from all participants. Participants were also asked to complete a number of questionnaires. Depressive symptoms were assessed using the Beck Depression Inventory [BDI] (Beck, Erbaugh, Ward, Mock, & Mendelsohn, 1961) which is a 21-item self-report multiple choice questionnaire. Neuroticism was assessed using the Eysenck Personality Questionnaire-Revised [EPQ-R] (Eysenck, Eysenck, & Barrett, 1985) consisting of 24 yes/no questions assessing neurotic behaviour. This measure was used to take into account participants with a high neurotic score which is also known to be a risk factor for depression (Kendler, Gatz, et al., 2006). The Spielberger State-Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) including 20 state and 20 trait anxiety questions was used to assess symptoms of anxiety. Sleep quality was

assessed using the 9-item Pittsburgh Sleep Quality Index [PSQI] (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and finally chronotype was measured using the 19-question Morningness-Eveningness Questionnaire [MEQ] (Horne & Ostberg, 1976a) that assesses an individual's preference for being alert in the morning or evening (or in between). As determined by Horne and Ostberg's cut-off scores for the MEQ, participants scoring 43 or above were determined to be early/intermediate chronotypes (EIC) and those with a score less than 43 were considered late chronotypes (LC).

### 2.2.3. Procedures

For all experiments E-Prime v2 (build 2.0.10.242, Psychology software tools) was used to design and present stimuli and record participant responses.

### 2.2.4. Emotional categorisation

Paradigms used for the emotional categorisation, recall and recognition experiments were adapted from a comparable set of paradigms developed by Harmer and colleagues (Harmer, Hill, Taylor, Cowen, & Goodwin, 2003). Participants were presented with 60 personality trait words (Anderson, 1968) on a computer screen for 500 ms each (3000 ms interstimulus interval) where half of the personality trait words were agreeable (e.g. generous) and half were disagreeable (e.g. lazy). Words were matched for length, frequency and meaningfulness. Participants were instructed to imagine overhearing another person describing them with the word and were asked to categorise the word as likeable or dislikeable, via keyboard press, as quickly and as accurately as possible. Mean accuracy and reaction time for agreeable and disagreeable words were computed.

### 2.2.5. Emotional recall and recognition

Without prior knowledge, and approximately 15 minutes after completion of the emotional categorisation task, participants were asked to recall as many of the agreeable and disagreeable personality trait words as possible (free recall). The number of correctly and incorrectly recalled words was recorded (ignoring reasonable spelling mistakes). Following this, participants were presented with 60 of the original agreeable/disagreeable personality trait words from the emotional categorisation task plus 60 (30 agreeable, 30 disagreeable) matched distractors on a computer screen. Participants indicated, via keyboard press, whether the presented words were ‘old’ (previously presented) or ‘new’ (distractor) to assess their recognition memory. Agreeable and disagreeable words were matched for length and frequency and presented in a random order with identical timings to the categorisation task (500 ms each, 3000 ms interstimulus interval) whilst response accuracy and reaction time were recorded.

### 2.2.6. Facial expression recognition

Two basic emotions (happiness and sadness) taken from four ‘characters’ featured in the NimStim series of facial expressions (Tottenham et al., 2009) were presented in the facial expression recognition task. All images of facial expression were displayed in greyscale and had been morphed in 10% steps from a neutral expression (0%) to the full prototypical expression (100%). A total of 84 stimuli were presented on the computer screen: 4 neutral stimuli, 40 examples of happy facial expressions and 40 examples of sad facial expressions (10 different intensities on 4 different characters for each emotion). Each stimulus was presented on the computer screen in a random order for 500 ms each interleaved with a blank screen. After the stimulus had been presented, participants were asked to indicate the emotional expression of the face (sad, neutral or happy) by clicking on a text box displayed at the bottom

of the screen. Participants were asked to respond as quickly and as accurately as possible. Mean recognition accuracy for each emotion (happy/sad) was calculated as well as for neutral faces and ambiguous faces (50% intensity sad and happy).

### 2.2.7. Dot probe task

Prototypical fearful, happy and neutral facial expressions were taken from the NimStim database (Tottenham et al., 2009) and presented in full colour and uncropped. Each emotional face was paired with a neutral facial expression (different character, same gender) so that there were 32 fear-neutral, 32 happy-neutral and 32 neutral-neutral pairs. Each trial began with a fixation cross presented in the centre of the computer screen for 2000 ms followed by a pair of faces either side of the fixation cross that were presented on the computer screen for 500 ms. Immediately after, participants were presented with a probe (asterisk) on either the left or right hand side of the screen where one of the images from the image pair was previously. The probe appeared to the left or right with equal frequency and the participant's response terminated the trial. Participants were asked to indicate, via key press, which side of the screen the probe had appeared on (left/right) as quickly and accurately as possible. Congruent trials were defined as trials where the probe replaced the emotional face and incongruent trials referred to the probe replacing a neutral stimulus. Vigilance scores for each participant were calculated by subtracting the median response time (excluding error trials) in the congruent trials from the response time in the incongruent trials. Hence, a higher vigilance score indicates a greater bias towards the emotional stimulus (see also *Table 1*).

## Balloon Analogue Risk Task (BART)

The Balloon Analogue Risk Task (BART) was used as a computerised measure of risk-taking/reward-seeking behaviour. Participants were instructed to inflate a cartoon balloon presented on the computer screen using keyboard press. On each trial, participants decided how many times to inflate the balloon which earned them 5 points each time. Participants had the option to ‘cash out’ at any time allowing the points to be banked or risk the balloon exploding where the points earned on that trial were lost (points earned on previous trials were unaffected). Participants were encouraged at the start of the experiment to earn the maximum number of points possible but were not given any information about the explosion parameters. A total of 40 trials were completed by each participant. The explode threshold for each trial was determined by randomly drawing a number from a uniform distribution that has a maximum value of 64. The probability that the balloon would explode after each additional pump within a trial was:  $P(\text{explode}) = 1/(64 - \text{number of pumps})$ , and therefore increased exponentially. Based on this, the maximum number of points would be earned by inflating the balloon 32 times per trial. For each individual, the mean number of adjusted pumps was computed i.e. the average number of pumps across trials where the balloon did not explode, similar to previous work (Lejuez et al., 2002). This overall measure of risk taking, extracted directly from the data, was chosen rather than absolute pump scores because it is not limited by trials where the balloon explodes which restricts the range of risky behaviour observed. Mathematical modelling techniques were also applied to the BART data in addition to adjusted pump scores to further explore risk-taking in relation to the learning and sequential choice processes involved (Wallsten, Pleskac, & Lejuez, 2005). The best-fitting model (Model 3) from Wallsten et al (2005) was chosen for this data which includes three estimated parameters: 1)  $\gamma^+$  - reward sensitivity, 2)  $\beta$  - response consistency, and 3)  $Var(q_1)$  - degree of uncertainty that the first balloon will not explode (see *Table 1*). Parameter values that best fitted the data were estimated

individually for each participant using MatLab (R2013a) maximum likelihood methods and the algorithm developed by Wallsten et al (2005).

*Table 1. Details of the outcome variables and their descriptions/interpretations for the Balloon Analogue Risk task and Dot probe task.*

<b>Experimental Task</b>	<b>Dependent Variable</b>	<b>Description</b>	<b>Interpretation</b>
<b>Dot probe task</b>	Vigilance score	Median response time to incongruent trials (neutral stimulus replaced by probe) minus median response time to congruent trials (emotional stimulus replaced by probe).	Higher scores represent greater bias to the emotional stimulus (happy/fearful facial expressions).
	Facilitation score	Response time to congruent trials minus response time to neutral pairs.	Faster response time to congruent trials represents a facilitation of attentional resources to that particular emotion (happy or fearful facial expressions).
<b>Balloon Analogue Risk Task (BART)</b>	Adjusted pumps	The mean number of pumps participants made on trials where the balloon did not explode.	A larger value indicates greater risky behaviour.
	$\gamma^+$	Reward sensitivity.	A measure of how much participants value the potential reward on a given trial based on prior experience. Higher values indicate greater sensitivity to reward and higher risk.
	$\beta$	Behavioural consistency.	Higher values represent deterministic response strategies whereas low values represent random choice.
	Var(q1)	Confidence in the initial perception of risk.	Small values represent greater confidence in the initial perception of risk.

*Note. Three of the measures ( $\gamma^+$ ,  $\beta$  and Var(q1)) are inferred from the data based on Wallsten and colleagues best fitting model (Wallsten et al., 2005).*

### 2.2.9 Time of testing

Participants completed all 6 experiments and questionnaires within a single session lasting between 1 and 1.5 hours. Sessions were scheduled for the convenience of the experimenter and participant but took place during working hours (08:30 to 18:30 hours). The start time of the experiment was recorded.

### 2.2.8. Statistical treatment

Statistical analyses were performed using SPSS version 24, plots were created in Microsoft Excel and R. Outliers on each task were determined to be response times that deviated +/- two standard deviations from the participant's mean and were removed from analyses. The data were first assessed for the assumptions of normality by applying the Shapiro-Wilk test. To explore participant demographics and trait characteristics, independent-samples t-tests and Chi-square tests for independence were used. Chronotype interaction effects were tested using a mixed-model 2-way analysis of variance (ANOVA) with Bonferroni correction where chronotype group (EIC/LC) was the between-subject factor and the within-subject factors were positive/negative stimuli in the following experiments: emotional categorisation, recognition, recall, facial expression recognition and dot probe. An independent samples t-test was used to determine differences in risk-taking scores during the BART task. Where necessary, time of experiment was added as a covariate in the analyses (ANCOVA). Interestingly, gender was found to significantly interact with time of experiment ( $F(1,90) = 15.25, p = .024, \eta_p^2 = .055$ ) such that female participants preferred attending experimental sessions earlier in the day. However, gender was not significantly different between chronotype groups and so was not added as a covariate in analyses to avoid collinearity effects. Secondly, to further explore the effect of chronotype in each experiment, correlations were performed using Spearman's



correlation coefficient. Partial correlations were performed to control for time of experiment and neuroticism (since later chronotype was moderately associated with higher neuroticism). The significance level was set at  $p < .05$ . P values between 0.051 and 0.07 were determined as a ‘trend’ towards statistical significance but are not discussed.

## 2.3. Results

### 2.3.1. Participants

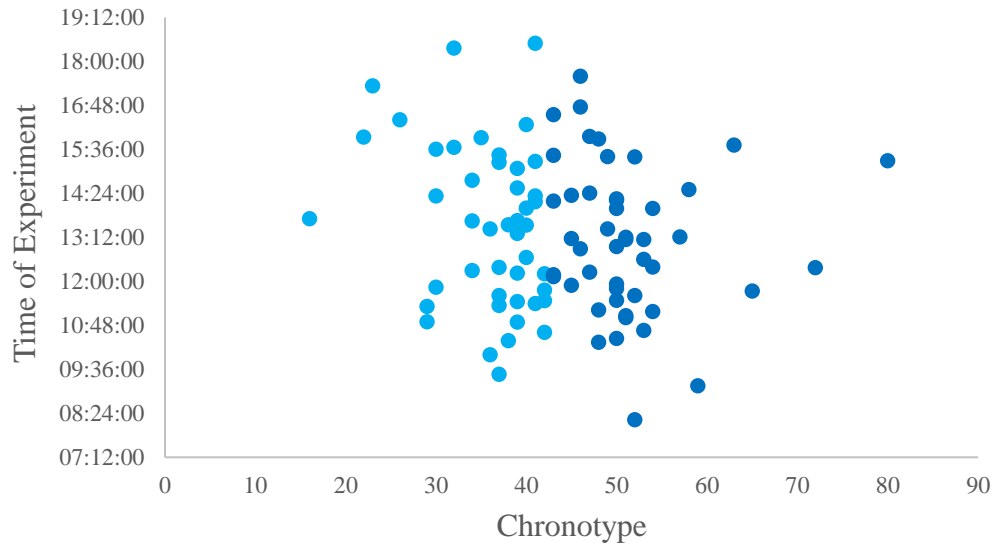
Participant characteristics are presented in *Table 2*. Forty seven participants considered themselves EIC ( $M = 51.20$ ,  $SD = 7.23$ , range 43 - 80) and 46 considered themselves LC ( $M = 35.74$ ,  $SD = 5.85$ , range = 16 – 42). Groups were similar in terms of age ( $t(91) = 1.08$ ,  $p = .28$ ), depressive symptoms ( $t(91) = -1.30$ ,  $p = .20$ ), state anxiety ( $t(91) = -.82$ ,  $p = .41$ ), trait anxiety ( $t(91) = -1.08$ ,  $p = .28$ ), sleep quality ( $t(89) = -.69$ ,  $p = .50$ ), neuroticism ( $t(78.12) = -1.94$ ,  $p = .057$ ), number of cigarettes smoked per week ( $t(87) = -.40$ ,  $p = .69$ ), units of alcohol consumed per week ( $t(87) = .31$ ,  $p = .76$ ) and gender ( $\chi^2(1) = .57$ ,  $p = .45$ ). Similarly, chronotype as a whole was not related to age, gender, depressive symptoms, state anxiety, trait anxiety, sleep quality, cigarette smoking or alcohol consumption ( $p > .05$ ). However, chronotype was weakly associated with neuroticism personality scores ( $r_s(92) = -.22$ ,  $p = .034$ ) such that later chronotype was associated with higher neuroticism.

No systematic bias was observed between the participant’s chronotype and the time of day the experiment was performed at (see *Figure 2*) such that EIC and LC participants were equally likely to attend morning or afternoon/evening sessions ( $t(91) = -.89$ ,  $p = .38$ ).

Table 2. Participant characteristics for early-intermediate (EIC) and late chronotype (LC) groups.

Measure	Chronotype		P-value	Range (this sample)	Range (original scale)
	EIC (n = 47)	LC (n = 46)			
Age	21.00 (5.20)	20.21 (1.88)	0.283	18 - 50	-
Gender F/M	37/10	39/7	0.450	-	-
Depressive symptoms	6.70 (5.35)	8.42 (8.18)	0.197	0 - 35	0 - 63
Neuroticism	9.22 (4.09)	11.28 (5.67)	0.057	0 - 24	0 - 24
State anxiety	34.80 (9.96)	36.33 (11.78)	0.413	20 - 72	20 - 80
Trait anxiety	37.63 (10.73)	40.07 (12.47)	0.284	20 - 77	20 - 80
Sleep quality	7.46 (3.67)	7.96 (3.18)	0.493	1 - 15	0 - 21
Number of cigarettes smoked per week	0.64 (2.32)	0.85 (2.58)	0.690	0 - 13	-
Number of alcoholic units consumed per week	2.66 (4.55)	2.42 (2.53)	0.763	0 - 15	-
Time of experiment	13:06	13:29	0.375	08:13 – 18:43	-

*Note. Values show mean (standard deviation). Depressive symptoms (Beck's Depression Inventory (BDI)), neuroticism (Eysenck's Personality Questionnaire – Revised (EPQ-R)), sleep quality (Pittsburgh Sleep Quality Index (PSQI)), state and trait anxiety (Spielberg's State-Trait Anxiety Inventory (STAI)). Also included is the questionnaire range (minimum – maximum) for the study sample and original scale.*



*Figure 2: Scatter plot showing no systematic bias between chronotype (early-intermediate chronotype = dark blue circles, late chronotype = light blue circles) and time of experiment.*

### 2.3.2. Emotional categorisation

#### *Accuracy:*

There was a significant main effect of valence on accuracy ( $F(1,90) = 9.30, p = .003, n_p^2 = .094$ ) where agreeable words ( $M = 87.46, SD = 12.42$ ) were more accurately categorised than disagreeable words ( $M = 84.09, SD = 11.10$ ) but this effect did not remain when time of experiment was added as a covariate (ANCOVA  $F(1,89) = .15, p = .70$ ). There was no significant main effect of chronotype or significant valence x chronotype interaction ( $F(1,90) = .39, p = .54$ ). Similarly, there was no significant correlation between chronotype and categorisation accuracy for agreeable ( $r_s(88) = -.057, p = .59$ ) or disagreeable ( $r_s(92) = .040, p = .71$ ) words.

#### *Reaction time:*

A significant main effect of valence on reaction time was observed ( $F(1,90) = 75.74, p < .001, \eta_p^2 = .457$ ) where reaction time for agreeable words ( $M = 787.96$  ms,  $SD = 15.11$  ms) was quicker than for disagreeable words ( $M = 882.00$  ms,  $SD = 21.19$  ms) but again this effect did not remain significant when the time of experiment was added as a covariate (ANCOVA  $F(1,89) = .16, p = .69$ ). There was no main effect of chronotype or significant valence x chronotype interaction ( $F(1,90) = .38, p = .54$ ). Similarly, there was no significant correlation between chronotype and categorisation reaction time for agreeable ( $r_s(88) = -.12, p = .31$ ) or disagreeable ( $r_s(88) = -.16, p = .14$ ) words.

### 2.3.3. Emotional recognition

#### *Accuracy:*

A significant main effect of valence on recognition accuracy was observed ( $F(1,88) = 19.14, p < .001, \eta_p^2 = .179$ ) where disagreeable words ( $M = 66.47, SD = 13.89$ ) were more likely to be accurately recognised than agreeable words ( $M = 62.66, SD = 13.13$ ). Again, this effect did not remain when time of experiment was added as a covariate (ANCOVA  $F(1,86) = .41, p = .52$ ). There was no main effect of chronotype or chronotype x valence interaction ( $F(1,88) = 1.62, p = .21$ ). Similarly, there was no significant correlation between chronotype and recognition accuracy for agreeable ( $r_s(84) = .18, p = .084$ ) or disagreeable ( $r_s(84) = .019, p = .87$ ) words.

#### *Reaction time:*

There was no significant main effect of valence ( $F(1,88) = .018, p = .89$ ) or chronotype ( $F(1,88) = 3.07, p = .083$ ) on recognition reaction time and no significant chronotype x valence

interaction ( $F(1,88) = .13, p = .74$ ) (Figure 3). However, there was a significant negative correlation between chronotype and recognition reaction time for agreeable words ( $r_s(84) = -.23, p = .030$ ) but not disagreeable words ( $r_s(84) = -.12, p = .26$ ) such that later chronotype was associated with an increased reaction time to recognise agreeable words (see Figure 3).

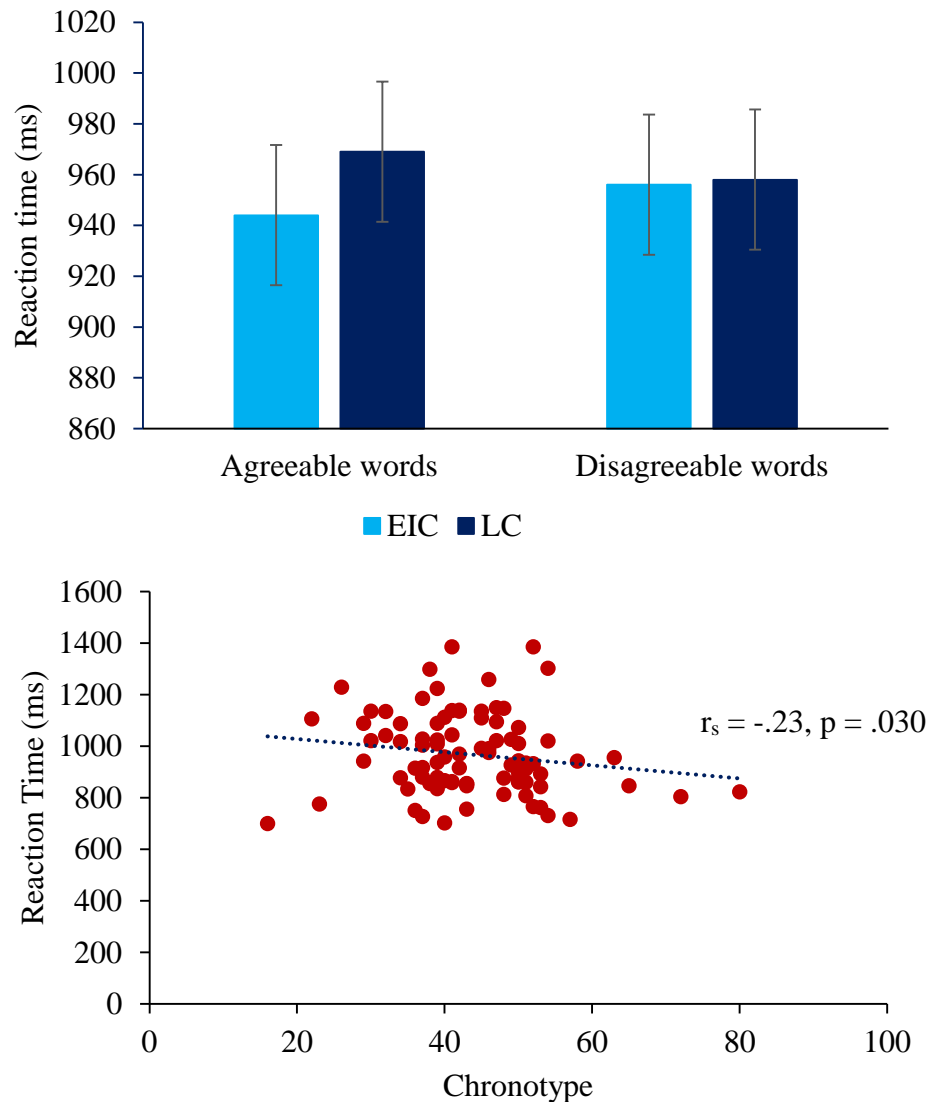


Figure 3. Top figure shows mean reaction time to accurately recognise agreeable and disagreeable words by EIC and LC groups during the emotional recognition task ( $p = ns$ ). Error bars show  $\pm$  standard error of the mean. Bottom figure shows significant relationship between recognition reaction time for agreeable words and chronotype with trend line.

### 2.3.4. Emotional Recall

#### *Accuracy*

For the emotional recall task, a significant main effect of recall accuracy was observed ( $F(1,88) = 11.43, p = .001, n_p^2 = .114$ ) where more agreeable words were freely recalled ( $M = 2.99, SD = 2.21$ ) compared to disagreeable words ( $M = 2.40, SD = 2.06$ ) and a significant main effect of chronotype was identified ( $F(1,88) = 6.22, p = .014, n_p^2 = .065$ ) where EIC recalled in total more words ( $M = 3.73, SD = 2.42$ ) than LC ( $M = 2.39, SD = 1.72$ ). There was also a significant chronotype x emotional recall interaction ( $F(1,88) = 3.83, p = .05, n_p^2 = .041$ ). Post-hoc t-tests revealed EIC types recalled more agreeable words ( $M = 3.73, SD = 2.41$ ) than LC ( $M = 2.39, SD = 1.72$ ) (independent-samples t-test  $t(89) = 3.06, p = .003$ ) but no differences were observed between groups for disagreeable words. EIC also recalled significantly more agreeable words than disagreeable words (paired t-test  $t(44) = 3.47, p = .001$ ) but this effect was not present in LC (see *Figure 4*). When time of experiment was added as a covariate, the main effect of chronotype (ANCOVA  $F(1,87) = 6.65, p = .012, n_p^2 = .071$ ) and recall accuracy (ANCOVA  $F(1,87) = 6.93, p = .01, n_p^2 = .074$ ) remained significant but the chronotype x accuracy interaction was non-significant (ANCOVA  $F(1,87) = 3.07, p = .083$ ). However, there was a significant positive correlation between chronotype and recall of agreeable words ( $r_s(85) = .22, p = .044$ ) but not disagreeable words ( $r_s(85) = .057, p = .60$ ) (see *Figure 4*) such that later chronotype was associated with reduced recall of positive words.

#### *Intrusions:*

There was a significant main effect of valence on number of intrusions ( $F(1,89) = 10.20, p = .002, n_p^2 = .10$ ) such that participants made significantly more intrusions for agreeable words ( $M = 0.35, SD = .06$ ) than disagreeable words ( $M = .12, SD = .03$ ) but this did not remain significant after time of experiment was controlled for (ANCOVA  $F(1,87) = .52, p = .47$ ). There

was no significant main effect of chronotype group or significant chronotype x valence interaction ( $F(1,89) = 2.88, p = .093$ ). Similarly, there was no significant correlation between chronotype and number of intrusions for agreeable ( $r_s(85) = .12, p = .25$ ) or disagreeable ( $r_s(85) = -.18, p = .097$ ) words.

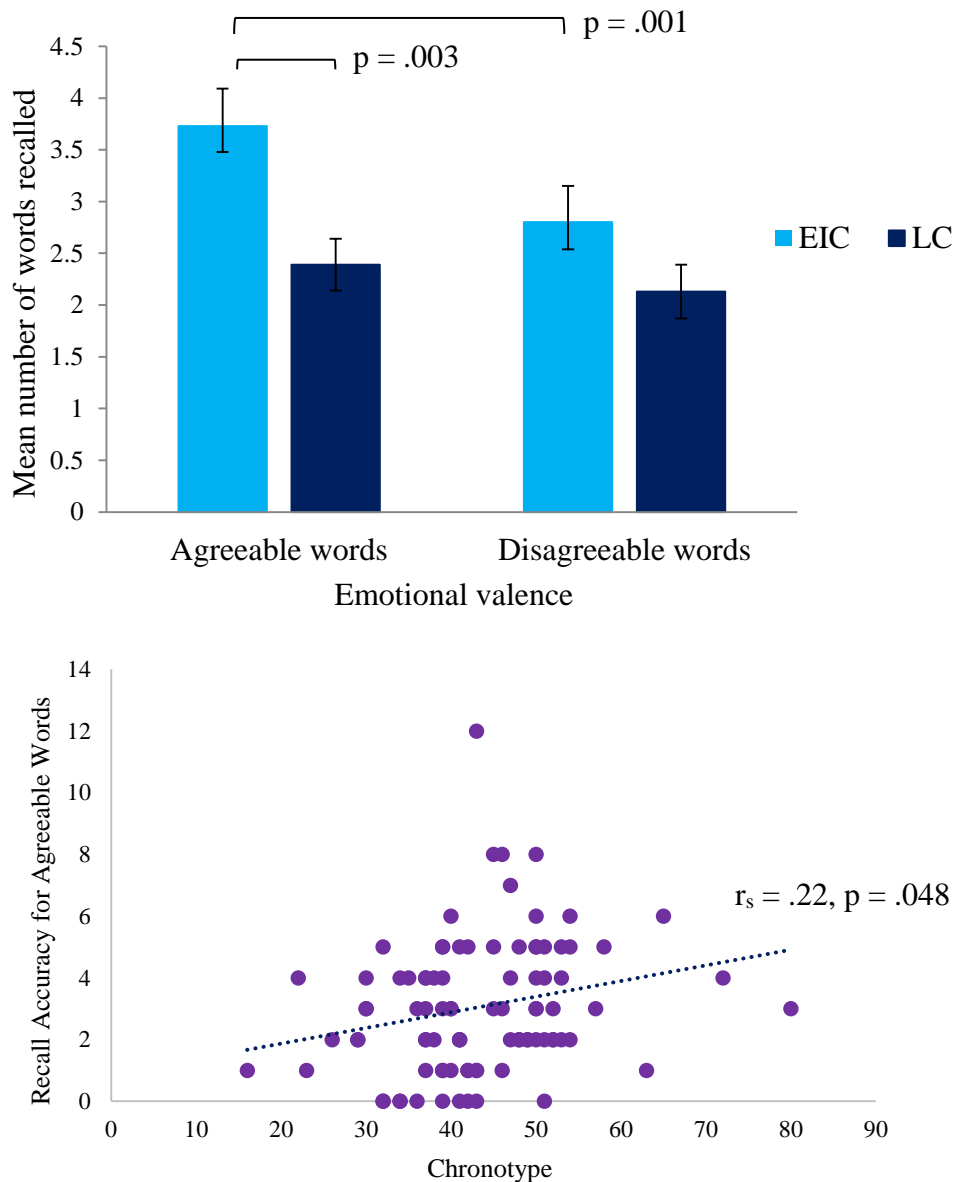


Figure 4. Top figure shows mean number of agreeable and disagreeable words accurately recalled by EIC and LC groups during the emotional recall task. Error bars show +/- standard error of the mean. Bottom figure shows recall accuracy for agreeable words across chronotype groups with trend line.

### 2.3.5. Facial expression recognition

A significant main effect of emotion was identified ( $F(1,77) = 16.50, p < .001, n_p^2 = .176$ ) where happy facial expressions ( $M = 67.56, SD = 11.15$ ) were more likely to be correctly identified than sad facial expressions ( $M = 62.75, SD = 12.15$ ). There was no significant main effect of chronotype ( $F(1,77) = 0, p = .99$ ). There was a significant chronotype x emotion interaction for recognition accuracy ( $F(1,77) = 3.93, p = 0.05, n_p^2 = .049$ ) where EIC individuals ( $M = 69.86, SD = 8.51$ ) accurately recognised more happy facial expressions than sad facial expressions ( $M = 67.33, SD = 7.91$ , paired samples t-test  $t(35) = 4.60, p < .001$ , see *Figure 5*) whereas this effect was not seen in LC individuals. This effect changed to a trend towards significance when time of experiment was added as a covariate ( $F(1,76) = 3.84, p = .054, n_p^2 = .048$ ). There was no significant relationship between chronotype and accuracy for happy ( $r_s(74) = .10, p = .39$ ) or sad ( $r_s(74) = -.11, p = .35$ ) faces.

In an exploratory analysis, there were no differences between chronotype groups in accurately recognising neutral faces (independent-sample t-test ( $t(68.2) = 1.37, p = .17$ )), ambiguous happy faces (50% intensity) ( $t(78) = 1.15, p = .25$ ) or ambiguous sad faces (50% intensity) ( $t(78) = -1.68, p = .097$ ).



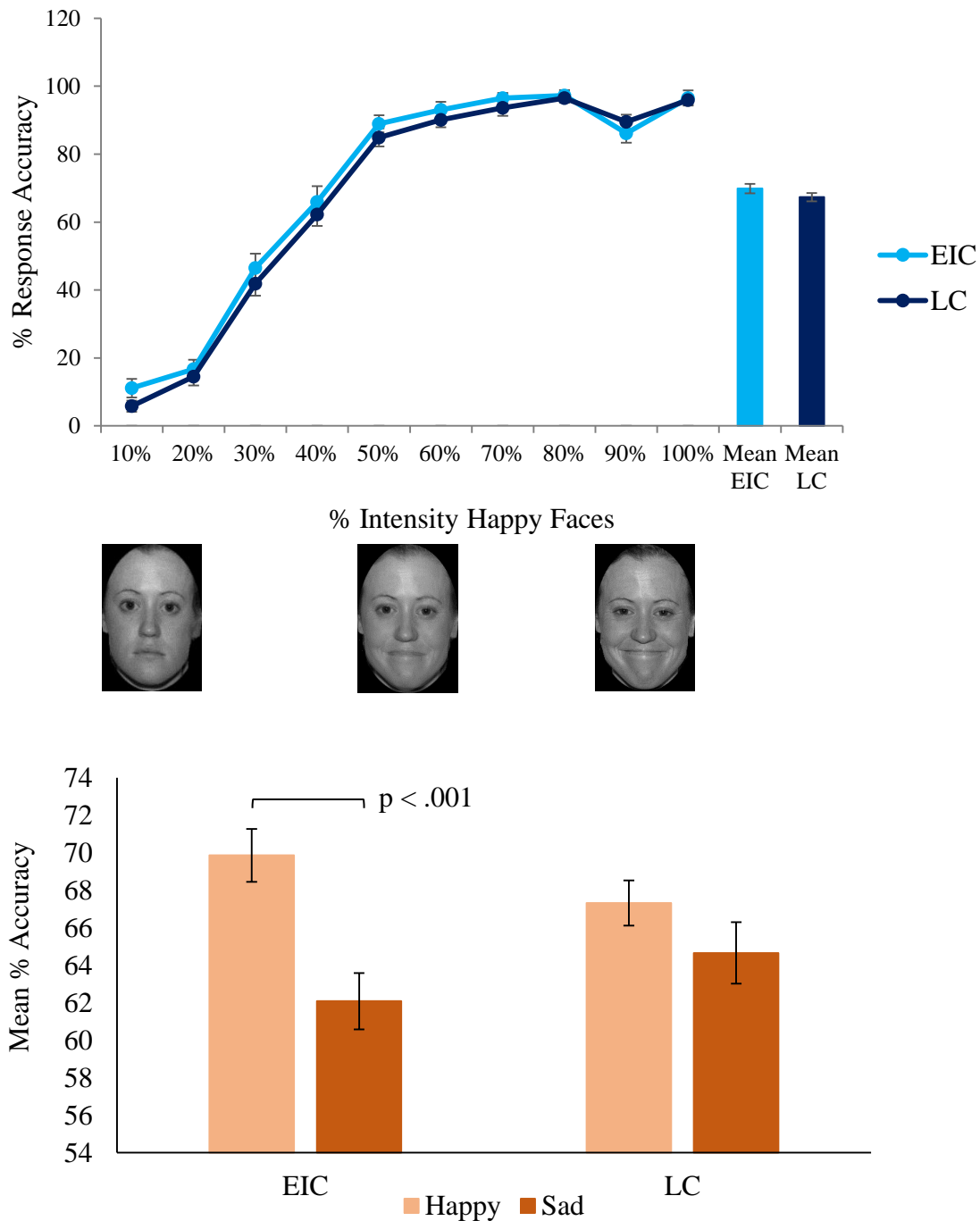


Figure 5. Top figure shows intensity-accuracy response curves to happy facial expressions for early-intermediate chronotype (EIC) and late chronotype (LC) groups. EIC response accuracy appears to be shifted to the left (relative to the LC group) suggesting this chronotype group are more accurate (or LC are less accurate) at recognising happy facial expressions. Also displayed are three example stimuli: neutral expression, 50% happy and 100% happy expression. Mean response accuracy for both groups is displayed on the right-hand side of the figure. Bottom figure shows mean response accuracy to happy and sad facial expressions between groups. Error bars represent standard error of the mean.

### 2.3.6. Dot probe task

For the dot probe task, there was no significant main effect of valence ( $F(1,78) = .18, p = .74$ ) or chronotype ( $F(1,78) = .82, p = .37$ ) on median vigilance score to fearful and happy facial expressions. Similarly, there was no significant interaction between chronotype group and valence ( $F(1,78) = .1, p = .75$ ) and no significant correlation between chronotype and vigilance scores for happy ( $r_s(874) = -.052, p = .65$ ) or fearful ( $r_s(74) = .11, p = .14$ ) facial expressions. There was also no significant interaction between chronotype group and facilitation score i.e. response time for neutral-neutral pairs subtracted from happy congruent pairs ( $F(1,76) = .11, p = .74$ ) or fearful congruent pairs ( $F(1,76) = 3.13, p = .081$ ).

### 2.3.7. Balloon Analogue Risk Task

The number of adjusted pumps (i.e. the average number of pumps on cash-out trials) was significantly different from the maximum number of pumps possible (64) for both EIC ( $t(35) = -18.39, p < .001$ ) and LC ( $t(42) = -16.52, p < .001$ ) groups suggesting that, on average, participants were risk-averse. The mean number of adjusted pumps did not differ between groups ( $t(77) = -.451, p = .65$ ). However, reward sensitivity ( $\gamma^+$ ) was significantly higher for EIC individuals ( $M = .72, SD = .41$ ) than LC individuals ( $M = .54, SD = .37, t(77) = 2.05, p = .044, n_p^2 = .052$ ) suggesting EICs valued the potential reward on given trials more based on prior experience. This effect was changed to a trend towards significance when time of experiment was added as a covariate (ANCOVA  $F(1,76) = 3.93, p = .051, n_p^2 = .049$ ). No significant differences were observed between groups for behavioural consistency ( $\beta$ ) or confidence in the initial perception of risk ( $\text{Var}(q1)$ ) ( $p > .05$ ) (see *Table 3*) and none of these measures (adjusted pumps,  $\gamma^+$ ,  $\beta$ ,  $\text{Var}(q1)$ ) were correlated with MEQ scores ( $p > .05$ ).

Interestingly, post-hoc analyses revealed that  $\gamma^+$  scores were significantly positively correlated with units of alcohol consumed per week for LC individuals (Spearman's correlation,  $r_s(43) = .39$ ,  $p = .009$ , see *Figure 6*) but not for EIC individuals. Simple linear regression showed that reward sensitivity was a significant predictor of alcohol consumption ( $\beta = .39$ ,  $p = .011$ ) for LC individuals such that 14.8% of the variance in alcohol consumption could be explained by degree of reward sensitivity.

*Table 3. Model parameters from the Balloon Analogue Risk Task (BART).*

Measure	Chronotype		P-value
	EIC (n = 36)	LC (n = 43)	
<b>Adjusted pumps</b>	15.19 (5.48)	15.8 (6.43)	0.653
<b>Behavioural Consistency (<math>\beta</math>)</b>	0.3 (0.23)	0.28 (0.15)	0.561
<b>Reward Sensitivity (<math>\gamma^+</math>)</b>	0.72 (0.41)	0.54 (0.37)	0.044 *
<b>Confidence in the Initial Perception of Risk (Var(q1))</b>	0.0003 (0.0005)	0.0024 (0.015)	0.391

*Note. Values show mean (+/- standard deviation) for chronotype groups (EIC = early-intermediate chronotype, LC = late chronotype). Also shown are independent samples t-test p-values comparing parameters between groups (\* =  $p < 0.05$ ).*

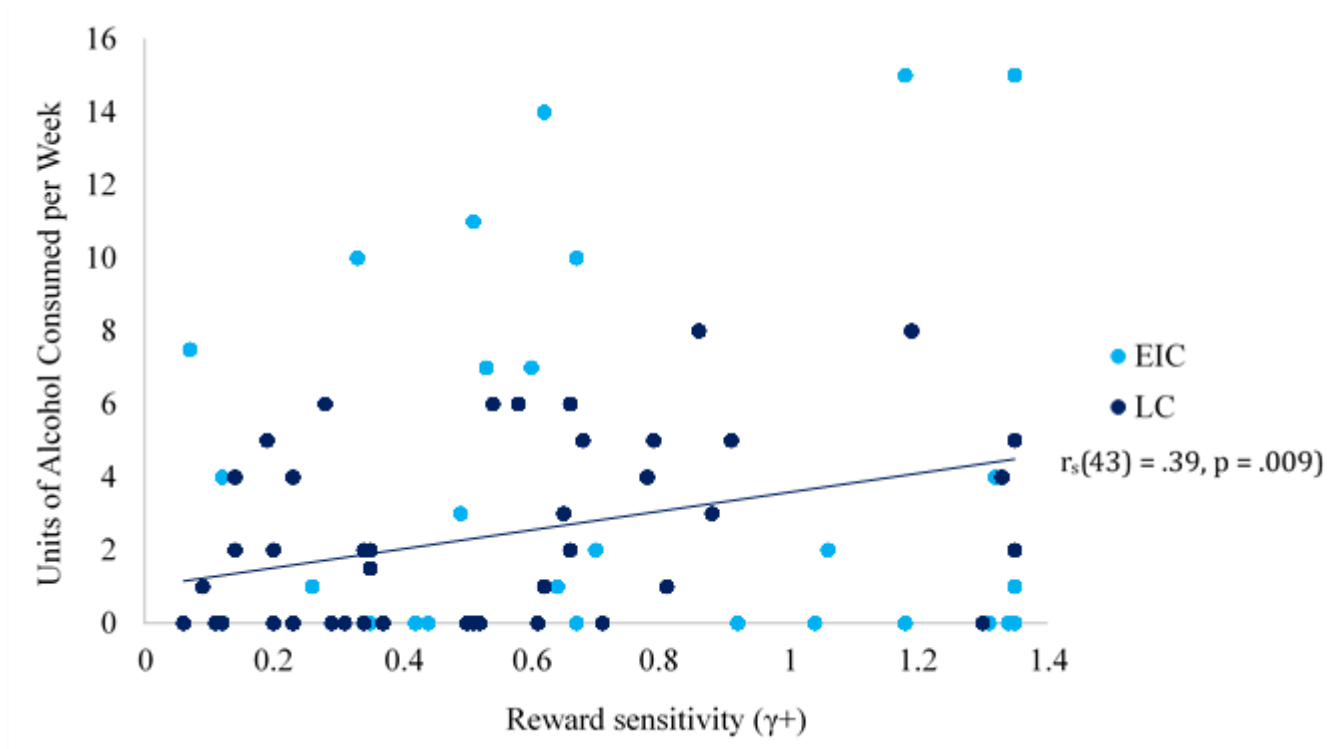


Figure 6. Scatter graph showing the relationship between participant's reward sensitivity score from the Balloon Analogue Risk Task (BART) and the units of alcohol consumed per week between EIC and LC groups. A significant correlation is present for LC individuals as shown by the trend line.

## 2.4. Discussion

The current findings suggest that emotional processing biases and altered reward sensitivity exist in healthy, LC individuals compared to EIC individuals. These differences were small but present despite groups being similar in terms of age, gender, depressive symptoms, anxious symptoms, sleep quality, cigarettes smoked and alcoholic units consumed, and were largely independent of synchrony effects (time of experiment) or neuroticism scores as these were controlled for in statistical analyses. Decreased positive or increased negative emotional

processing was found across several tasks including the emotional recognition, emotional recall and facial expression recognition tasks and altered reward sensitivity was observed in the BART task. In contrast, there was no evidence that attentional bias (measured using dot-probe task) or some forms of self-referent biases (emotional categorisation) were different between chronotype groups. Converging evidence suggests late chronotypes are at increased risk for developing depression (Hidalgo et al., 2009; Merikanto et al., 2013, 2015) and here, late chronotypes display emotional biases and altered reward sensitivity similar to those reported in depressed individuals (Bradley et al., 1995; Peckham et al., 2010) remitted depressed patients (Anderson et al., 2011) and at-risk populations (Chan et al., 2007) which are thought to be important for the aetiology and maintenance of depression (Beck et al., 1979). Therefore, negative emotional biases and altered reward sensitivity may represent trait vulnerability markers for depression in late chronotype individuals.

The current data highlight a number of interesting findings relating to previous studies involving depressed patients and similar healthy, at-risk populations. For example, later chronotype was associated with reduced recall of agreeable personality trait words and a slower reaction time to correctly recognise the same agreeable words, suggesting decreased emotional processing of positive information. Since participants were asked to process the words in relation to themselves, the findings suggest that negative biases were mostly related to self-referent processing although no control condition was included. Both findings were better revealed across the whole sample (instead of between EIC/LC groups) suggesting the more extreme chronotypes were driving this effect. These findings partially replicate those reported by Berdynaj and colleagues showing EIC types were significantly better at recalling and recognising positive personality trait words than negative words whereas LC types showed no difference (Berdynaj et al., 2016). Similarly, reduced recall, recognition and categorisation of positive information has been robustly reported in depression (Bradley et al., 1995) and in a

number of at-risk populations including individuals with high neuroticism (Chan et al., 2007), familial risk for depression (Mannie, Bristow, et al., 2007), a negative cognitive style (Alloy et al., 1997) and with subclinical depressive symptoms (Connolly et al., 2016). It is hypothesised that depressed individuals have negative self-schemas that facilitate faster responses to negative self-referent adjectives, or slower responses to positive self-referent adjectives, as these decisions are considered more congruent with their self-concept. Similarly, mood-congruent information is thought to be encoded more deeply in depression and therefore negative information is better retrieved (Matt et al., 1992). This suggests that late chronotypes have similar negative self-schemas to depressed patients which could underlie their increased risk for the disorder.

Interestingly, LC individuals recalled significantly fewer words overall than EICs despite controlling for possible synchrony effects (time of experiment). This was an unexpected finding but may relate to the general memory impairments observed in depressed patients. For example, a meta-analysis of 40 studies of depression found significant cognitive deficits across a range of memory tasks although there is inconsistency regarding different types of memory which could be related to participant characteristics (e.g. age, education etc.) (Kindermann & Brown, 1997). Generally, explicit memory has been shown to be most affected in depression, especially on effortful tasks (e.g. free recall) as reported here, compared to automatic tasks (e.g. recognition) (Roy-Byrne, Weingartner, Bierer, Thompson, & Post, 1986). Indeed, cognitive deficits in depression have been linked to a lack of motivation which is a defining symptom of the disorder (Austin, Mitchell, & Goodwin, 2001). Moreover, Roeser and colleague's (2013) Academic Performance Model indicated that the relationship between chronotype and academic performance was mediated by motivation such that later chronotype was associated with poorer learning motivation (Roeser, Schlarb, & Kübler, 2013). There is also (limited) evidence that global cognitive impairments represent a trait marker for depression in other at-

risk populations. For example, Mannie and colleagues (2009) reported that young healthy women with a family history of depression displayed decreased immediate recall (and recognition) compared to controls, and this was associated with increased salivary cortisol measures (Mannie, Barnes, Bristow, Harmer, & Cowen, 2009). Therefore, future studies probing global cognitive deficits and motivation in LC individuals whilst specifically controlling the time of experiment are needed to investigate this further.

Marginally significant group differences were also observed in the facial expression recognition task; specifically, EIC individuals accurately identified significantly more happy facial expressions than sad faces whereas this difference was not observed in LC individuals. Similar to the emotional recall and recognition tasks, this finding suggests reduced positive information processing in this at-risk group. Negative biases towards facial expressions have been widely reported in depression (Bouhuys et al., 1999; Sloan et al., 2001) and in other at-risk populations (Chan et al., 2007; Gibb et al., 2009; Joormann et al., 2010; Lopez-Duran et al., 2013) and are thought to be specifically related to negative interpretations of social interactions. In depressed patients (Bradley & Mathews, 1983; Bradley, Mogg, Millar, & White, 1995; Denny & Hunt, 1992; Derry & Kuiper, 1981) and in some studies of at-risk individuals (Dainer-Best et al., 2018; Taylor & Ingram, 1999), decreased processing of positive stimuli is often accompanied by increased processing of negative stimuli such as recalling significantly more negative words. Chan and colleagues (2007) suggested that decreased positive processing in vulnerable individuals may later evolve into additional increased negative processing in the depressed state (Chan et al., 2007). The current findings (recall, recognition and facial recognition tasks) suggest this hypothesis could be extended to LC individuals, although Berdynaj and colleagues (2016) reported that LC individuals accurately identified significantly more sad faces than EIC individuals suggesting a further processing bias towards negative stimuli.

In contrast, there was no evidence for effects of late chronotype on self-referent categorisation or attentional biases (vigilance to emotional expressions). There is conflicting evidence for the existence of attentional biases in depression. Some studies have reported attentional biases towards negative information in individuals with depression (Peckham et al., 2010), children at genetic risk of depression (Joormann et al., 2007; Kujawa et al., 2011) and healthy controls with induced depression (Bradley et al., 1997), whilst others have not (Chan et al., 2007; Hill & Dutton, 1989). Berdynaj and colleagues (2016) also reported a positive attentional bias towards happy facial expressions in EIC individuals compared to LC individuals (Berdynaj et al., 2016). The conflicting findings might be explained by experiment timing. Compared to Berdynaj et al (2016), there was no systematic bias between time of experiment and chronotype group and time of experiment was further controlled for in statistical analyses. In a recent study by Barclay and Myachykov (2017), later chronotype was associated with slower reaction times on the Attention Network Task when tested at 2am compared to 8am, whereas early chronotype exhibited an opposite pattern, suggesting an asynchrony effect i.e. performance better at non-optimal time of day (Barclay & Myachykov, 2017). Alternatively, higher depressive and anxious symptoms are reported in this sample compared to Berdynaj et al's sample. It has been reported that individuals with Generalised Anxiety Disorder (GAD) show greater colour-naming interference effects on the Stroop task compared to individuals with GAD and a concurrent diagnosis of depression (Bradley et al., 1995). Moreover, stimulus timing has been shown to reveal attentional biases in different populations. For example, subliminal attentional biases (e.g. 50 ms) have been more robustly reported in anxious patients whereas more consistent findings in depression have been reported when stimuli are presented for longer e.g. 1 second or more (Mogg, Bradley, & Williams, 1995a), compared to medium lengths of time e.g. 500 ms as in this study, where findings seem to be mixed (Berdynaj et al., 2016; Chan et al., 2007; Mathews et al., 1996). Therefore, it may



be that the increased depression and anxiety scores in our sample mask the attentional bias. Further studies better controlling for time of experiment and varying stimulus presentation timing are warranted.

As well as evidence for emotional biases in relation to late chronotype, computational modelling revealed that LC individuals were less sensitive to potential rewards on a given trial based on previous experience and therefore adjusted their risk behaviour less often compared to EIC individuals. A reduction in reward sensitivity has also been observed in studies of depression (Hevey et al., 2017) and some studies of at-risk individuals including; high neuroticism (Denburg et al., 2009) and increased familial risk for depression (Mannie et al., 2015) showing impaired performance on risk-taking tasks including the BART. As noted in Chapter 1, depression has been associated with a blunted response to reward and is consistent with symptoms of anhedonia (Eshel & Roiser, 2010). Deficits in the reward system have also been linked to reduced motivation in depression which results in a ‘conservative response bias’ i.e. patients require a greater degree of certainty before responding (Henriques & Davidson, 2000). As noted above, later chronotype has been associated with a lack of learning motivation which could be related to poorer reward sensitivity observed here as well. In contrast, Hasler and colleagues (2013) reported that late chronotype was associated with reduced medial prefrontal cortex (mPFC) reactivity and enhanced ventral striatum reactivity during a monetary reward task using fMRI suggesting reduced regulatory control and elevated reward sensitivity (Hasler, Sitnick, Shaw, & Forbes, 2013). It is unknown how this altered neural activity relates to behavioural measures of risk and reward but the authors suggested that altered reward function associated with late chronotype could lead to opposite trajectories i.e. anhedonia/depression or impulsivity/sensation-seeking. Therefore, the current findings provide evidence that impairments of the reward system exist prior to the onset of depression in late chronotype individuals, which could be related to reduced motivation. Interestingly, post-hoc

analysis of the BART task also revealed that for LC individuals, reward sensitivity was a significant positive predictor of alcohol consumption despite no differences in alcohol consumption between chronotype groups. Previous studies have indicated that increased reward sensitivity is a significant predictor of alcohol consumption (Jonker, Ostafin, Glashouwer, van Hemel-Ruiter, & de Jong, 2014) and late chronotype has been associated with greater self-reported general risk-taking (Ponzi, Wilson, & Maestriperi, 2014), impulsivity and sensation-seeking (Kang et al., 2015). There is also some evidence that a link between late chronotype and alcohol dependence is indirectly mediated by mPFC reactivity to reward (Hasler, Casement, Sitnick, Shaw, & Forbes, 2017). Therefore, future studies investigating how these factors (chronotype, alcohol consumption and reward sensitivity) interact are warranted.

In contrast, no significant differences in overall risk-taking (adjusted pumps) were observed in the current data. It was predicted that chronotypes would show increased risk-taking on this task which may be related to their reduced reward sensitivity. However, this finding is consistent with two other studies of chronotype and risk-taking (Berdynaj et al., 2016; Killgore, 2007) and is partly consistent with Ingram and colleague's (2016) findings showing no differences in adjusted pumps between early chronotype (EC) and LC groups when chronotype was measured using the MEQ. In these studies (and as noted above), LC individuals self-reported a higher propensity to take risks but displayed no evidence for increased risk-taking using the BART (Killgore, 2007). This suggests that there is a difference between self-reported risky behaviours and behavioural measures of risk-taking. Indeed, the BART has only been moderately correlated with real-world risk-taking behaviours including gambling, drinking alcohol and being involved in a physical fight (Lejuez, Aklin, Zvolensky, & Pedulla, 2003). One explanation for this mismatch may be that more sensitive measures of risk-taking (such as reward sensitivity as reported here) are needed as there may be a more complicated relationship between risk-taking behaviour and altered reward sensitivity in relation to late chronotype

(highlighted above). Indeed, many studies report an overall measure of risk-taking e.g. adjusted pumps or risk/benefit ratio, rather than specific measures of reward and punishment sensitivity. Another explanation may be that different measurements of chronotype and times of experiment are reported between studies. For example, when chronotype was measured using genotyping, Ingram and colleagues reported that EC individuals took significantly more risks (increased adjusted pumps) on the BART task, compared to LC individuals, and took significantly more risks when they were tested later in the day (Ingram et al., 2016). This suggests that risky decision-making may be affected by synchrony effects and may differ depending on the measurement of chronotype used (genotyping vs. MEQ). Therefore, future studies of chronotype should focus on more sensitive behavioural measures of risk-taking as well as exploring the effects of chronotype measurement and time of experiment more closely.

#### 2.4.2. Specific limitations

There are several specific limitations that should be considered when interpreting the current findings (although more general limitations relating to all experimental chapters are discussed in Chapter 9). Firstly, due to the relatively limited sample size and difficulty in recruiting the more extreme chronotypes (particularly EC), chronotype was dichotomised into two groups according to pre-defined cut-off scores, similar to previous studies (e.g. Berdynaj et al, 2016). Early chronotypes and intermediate chronotypes were also collapsed into a single group (EIC). This was likely to increase the variance within groups and obscure the full range of emotional processing biases in relation to chronotype. Future experimental chapters have aimed to overcome this limitation by either; recruiting larger sample sizes in order to establish three chronotype groups, or treating chronotype as a continuous variable and pre-screening participants for extreme chronotypes (neuroimaging chapters). Secondly, emotional categorisation, recall and recognition tasks required participants to process positive and

negative personality trait words as self-referential but this was not confirmed by participants or compared to a control condition. Therefore, negative biases observed in these tasks may not be wholly related to self-referential processing. Thirdly, there was a strong trend for a group difference in neuroticism between chronotype groups ( $p = .057$ ). Although this difference was not statistically significant, high neuroticism may have been a contributing factor towards the current findings since emotion processing biases have been previously reported in highly neurotic participants (e.g. Chan et al. 2007). In additional analyses where chronotype was treated as a continuous variable, neuroticism was added as a covariate and future studies should aim to account for this factor. Finally, although statistical analyses were performed with post-hoc Bonferroni correction, a number of additional exploratory analyses (e.g. correlations) were performed where multiple comparisons were not corrected for. Therefore, these findings should be interpreted cautiously.

## 2.5. Conclusion

In conclusion, the current findings suggest that alterations in emotional processing and reward processing are present in LC individuals that are at increased risk of developing depression but have never been depressed. These biases present themselves across a range of tasks including emotional recall and recognition, facial expression recognition and the BART but are not present in dot-probe or emotional categorisation tasks. Here, LC individuals display a biased response away from positive emotional stimuli which may in turn develop into a biased response towards negative information should depression be triggered in these individuals. LC individuals also displayed decreased reward sensitivity indicating a blunted reward system, consistent with symptoms of anhedonia in depression. As outlined in Beck's (1967) cognitive theory of depression, negative cognitive biases play a key role in the development of

depressogenic thoughts and beliefs in vulnerable individuals. Negative interpretations of the environment and social interactions in turn allow negative cognitive biases to persist and the depressed state to be maintained. Hence, these results provide support that cognitive biases exist prior to the onset of depression in late chronotype individuals and could represent a trait vulnerability marker for the disorder.

# CHAPTER THREE

## Facial Expression Recognition

### 3.1. Introduction

As reported in Chapter 2, there was a significant chronotype x valence interaction for the facial expression recognition task such that early-intermediate chronotype (EIC) individuals recognised significantly more happy facial expressions than sad expressions whereas no differences were reported for late chronotype (LC) individuals. Similarly, Berdynaj et al (2016) reported that LCs had enhanced accuracy to identify sad faces compared to EICs but no differences were observed for happy facial expressions (Berdynaj et al., 2016). This pattern of results suggests an emotional bias towards negative stimuli and/or away from positive stimuli in LC individuals which is similar to that seen in both acutely depressed (Bouhuys et al., 1999; Ekman & Friesen, 1971; Feinberg et al., 1986; Flanagan et al., 2011; Gur et al., 1992; Sloan et al., 2001; Surguladze et al., 2005), remitted depressed patients (Anderson et al., 2011) and other populations at increased risk for depression (Chan et al., 2007; Gibb et al., 2009; Joormann et al., 2010; Lopez-Duran et al., 2013). Cognitive theories of depression emphasise the role of negative emotional biases in the aetiology and maintenance of depression (Beck, 1989) and biases towards facial expressions are thought to be specifically related to negative interpretations of social interactions. There is increasing evidence that LCs are at increased risk for developing depression and negative emotional biases towards facial expressions could represent a psychological mechanism underpinning this risk.

A significant limitation, however, of the findings reported in Chapter 2 and in Berdynaj et al (2016) was the collapsing of early and neither chronotype participants into a single group

(EIC). Moreover, time of experiment was not controlled for in the analyses by Berdynaj and colleagues (2016) so possible (a)synchrony effects cannot be ruled out. The current study, therefore, aims to replicate and extend these earlier findings by exploring expression recognition in a large sample of participants stratified into; early-, neither- and late-chronotype individuals. Two hundred and twenty-six individuals completed an online survey including measures of sleep quality, depression/anxiety and chronotype followed by a similar facial expression recognition task. It was hypothesised that, similar to depressed patients and previous work (Berdynaj et al., 2016), late chronotypes will show increased accuracy to identify sad facial expressions and/or decreased accuracy to identify happy facial expressions.

## 3.2. Methods

### 3.2.1. Participants

A total of 315 participants completed the online study and were recruited via email advertisement, social media or personal contact. Of these, 89 participants were excluded due to reporting one or more co-variables; a diagnosis of depression (79), sleep disorder (15), PHQ\_4 score > 9 (19) or under the age of 18 (2). Hence, a total of 226 participants were included in the final analyses (see *Figure 7* for break-down of chronotype groups). The study was approved by the local ethics committee and informed consent was obtained prior to any study procedures taking place.

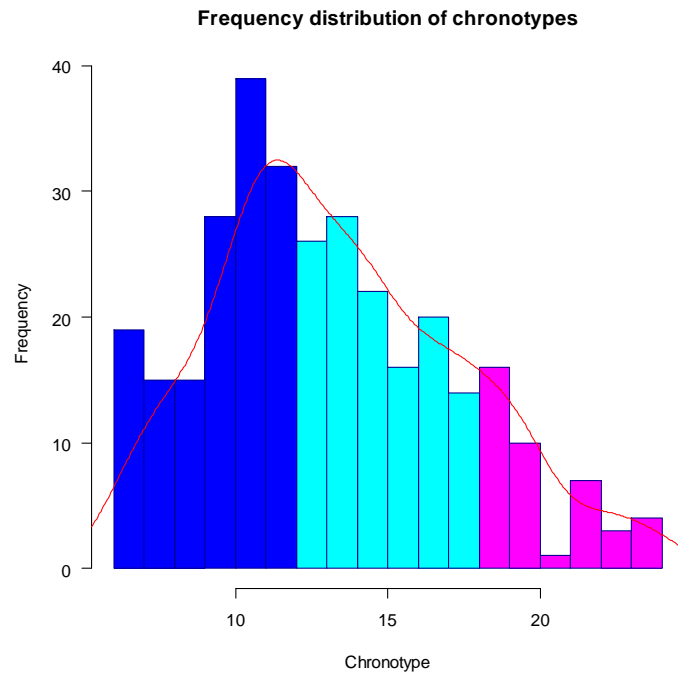


Figure 7. Frequency distribution of chronotypes ( $n = 226$ ). Dark blue shows late chronotypes (LC), light blue shows neither chronotypes (NC) and pink shows early chronotypes (EC) as defined by Horne and Ostberg (1976).

### 3.2.2. Procedures

Participants completed an online survey including a facial expression recognition task (please see below) and three questionnaires: the Pittsburgh Sleep Quality Index [PSQI], the Patient Health Questionnaire [PHQ-4], and the reduced Morningness-Eveningness Questionnaire [rMEQ]. The Pittsburgh Sleep Quality index [PSQI] (Buysse et al., 1989) is a 19-item questionnaire used to measure sleep quality over the previous month using a 0 – 3 interval scale. Here, global PSQI scores are reported with lower scores indicating better sleep quality. The Patient Health Questionnaire [PHQ-4] (Löwe et al., 2010) is an ultra-brief (4 questions) screening tool for anxiety and depression. Finally, the reduced Morningness-Eveningness Questionnaire [rMEQ] (Adan & Almirall, 1991b) is a 5-item questionnaire used to assess participants' chronotype – participant's with an rMEQ score of 18 or above were determined to be early chronotypes (EC), those with a score between 12 – 17 were considered



neither chronotypes (NC) and participants with a score  $\leq 11$  were deemed late chronotypes (LC). The shorter version of the MEQ was used here in order to mitigate demands on participants and encourage completion of the study. The rMEQ has been shown to be highly correlated with the full version of the MEQ (Adan & Almirall, 1991a) and has good external validity (Vincenzo, José, Monica, & Marco, 2006).

### 3.2.3. Facial expression recognition

The facial expression recognition task featured two basic emotions (happiness and sadness) taken from four individual characters included in the NimStim series of facial expression ([www.macbrain.org/resources.htm](http://www.macbrain.org/resources.htm)). All images were presented in greyscale and had been morphed between each prototypical and neutral expression in 10% steps (0% = neutral, 100% = full emotion). Four examples of each emotion at each intensity were presented (two emotions x ten intensities x four examples = 80 stimuli). Each face was also presented in a neutral expression (0% = neutral expression, four stimuli), giving a total of 84 stimuli presentations. Stimuli were presented in random order and participants indicated their responses by selecting either SAD, NEUTRAL or HAPPY on the screen. The response rule was counterbalanced across stimuli. The number of stimuli accurately classified at each emotion was recorded.

### 3.2.4. Time of testing

Participants were free to complete the experiment and questionnaires online at any time of the day and the start time of the experiment was recorded.

### 3.2.5. Statistical treatment

IBM SPSS v24 Statistics Software package was used to analyse the data. The data were first assessed for the assumptions of normality by applying the Shapiro-Wilk test. One-Way Analysis of Variance (ANOVA) and Pearson's chi-square test for independence were used to explore participant demographics and trait characteristics. For the facial expression recognition task, analyses were conducted using mixed-model two-way ANOVA with Bonferroni correction where chronotype (MT, NT and ET) was the between-subjects factor and facial expression valence (happy, sad) was the within-subjects factor. Where necessary, age and time of experiment were added as covariates in the analyses (ANCOVA) to control for differences in age between groups and possible (a)synchrony effects. Secondly, to further explore the effect of chronotype in each experiment, correlations were performed using Spearman's correlation coefficient. Partial correlations were performed to control for time of experiment and sleep quality (since later chronotype was moderately associated with poorer sleep quality). The significance level was set at  $p < .05$ .

## 3.3. Results

### 3.3.1. Participants

Demographic details of the study population according to chronotype are shown in *Table 4*. Based on the rMEQ questionnaire scores, 45 participants considered themselves early chronotypes [EC] (M 19.96, SD 1.80, range 18-24), 114 neither chronotype [NC] (M 14.18, SD 1.73, range 12-17) and 67 late chronotypes [LC] (M 9.63, SD 1.39, range 6-11). Chronotype groups were similar in terms of sleep quality ( $F(2,225) = .79, p = .43$ ), depression and anxiety ( $F(2,223) = 2.92, p = .056$ ). Morning types were older than both NT and ET groups ( $F(2,223)$

= 9.60,  $p < .001$ ) and a greater number of individuals in the NC group were female ( $\chi^2(2,226)$ ,  $p < .05$ ) (*Table 4*). Secondly, later chronotype was not significantly related to age, gender or depressive/anxious symptoms but was significantly related to poorer sleep quality ( $r_s(226) = -.16$ ,  $p = .019$ ).

*Table 4. Sample characteristics according to chronotype.*

	<b>Chronotype</b>		
	Early chronotype (EC) ( $n = 45$ )	Neither chronotype (NC) ( $n = 114$ )	Late chronotype (LC) ( $n = 67$ )
<b>Age</b>	36.93 (16.92)	27.71 (11.97)	27.28 (11.11)
<b>Gender (M/F)</b>	21/24	30/84	25/42
<b>Depressive/anxious symptoms</b>	1.44 (2.03)	2.37 (2.20)	2.16 (2.23)
<b>Sleep quality</b>	4.84 (3.10)	5.48 (3.11)	5.42 (2.67)
<b>Time of experiment</b>	15:24 (4.36)	15:42 (4.44)	15:14 (5.47)

*Note. Data (except gender) are mean (SD). Sleep quality (PSQI - Pittsburgh Sleep Quality Index); depressive/anxious symptoms (PHQ \_4 - Patient Health Questionnaire).*

No systematic bias was observed between the participant's chronotype and the time of day the experiment was performed at (see *Figure 8*) such that EC, NC and LC participants were equally likely to complete the experiment in the morning/afternoon/evening ( $F(2,223) = .20$ ,  $p = .82$  and no significant relationship was observed between rMEQ score and timing ( $r_s(226) = .006$ ,  $p = .93$ ).

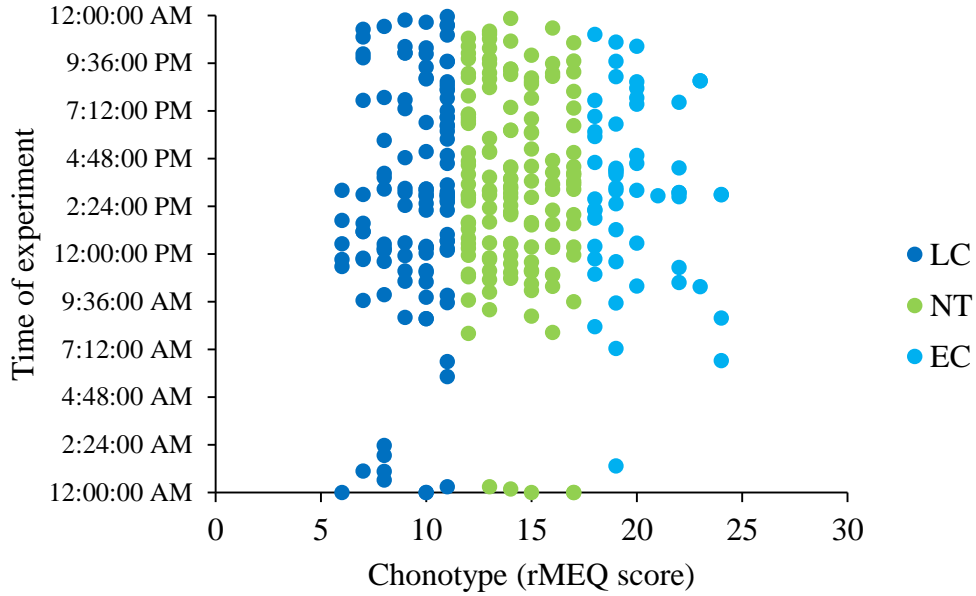
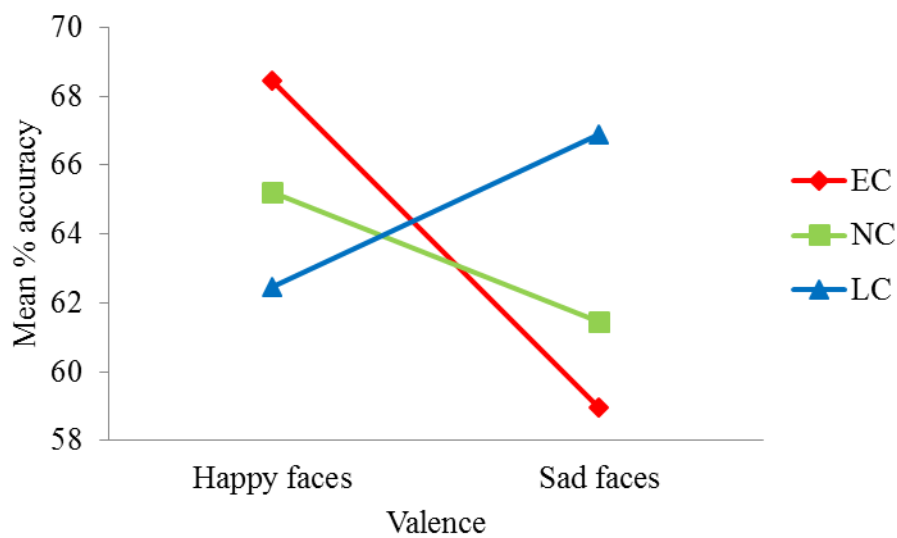


Figure 8. Scatterplot showing no systematic bias between chronotype and the time at which the experiment was completed at. *rMEQ* = reduced Morningness-Eveningness Questionnaire.

### 3.3.2. Facial expression recognition

There was a significant main effect of valence on recognition accuracy ( $F(1,223) = 7.86$ ,  $p = .005$ ,  $\eta_p^2 = .034$ ) but no main effect of chronotype. There was a significant group x emotion interaction ( $F(2,223) = 13.05$ ,  $p < .001$ ,  $\eta_p^2 = .105$ ) (see Figure 9) and simple effects analyses for each emotion indicated that the means for the three chronotype groups differed for sad faces ( $F(2,223) = 6.17$ ,  $p = .002$ ,  $\eta_p^2 = .052$ ) and showed a trend towards significance for happy faces ( $F(2,223) = 3.00$ ,  $p = .052$ ,  $\eta_p^2 = .026$ ). When age and time of experiment were added as covariates, the main effect of valence did not remain but chronotype x valence interaction remained significant ( $F(2,221) = 11.84$ ,  $p < .001$ ,  $\eta_p^2 = .097$ ). Post hoc analyses, with Bonferroni correction, revealed that LC individuals recognised significantly more sad faces

(mean= 66.90, SD= 13.71) than EC individuals ( $M= 58.94$ ,  $SD= 13.48$ ,  $p = .004$ ) and NC individuals ( $M= 61.47$ ,  $SD= 11.73$ ,  $p = .018$ ) (*Figure 10*). Furthermore, LCs recognised significantly fewer happy facial expressions than EC ( $M = 62.46$ ,  $SD= 12.59$  vs.  $M= 68.44$ ,  $SD= 14.67$ ,  $p = .046$ ) but not NC individuals. Similarly, later chronotype was significantly related to better recognition of sad facial expressions ( $r_s(222) = -.21$ ,  $p = .001$ ) and poorer recognition of happy facial expressions ( $r_s(222) = .17$ ,  $p = .011$ ).



*Figure 9. Graph showing significant chronotype  $\times$  valence interaction on mean recognition accuracy for happy and sad facial expressions. EC = early chronotype, NC = neither chronotype, LC = late chronotype.*

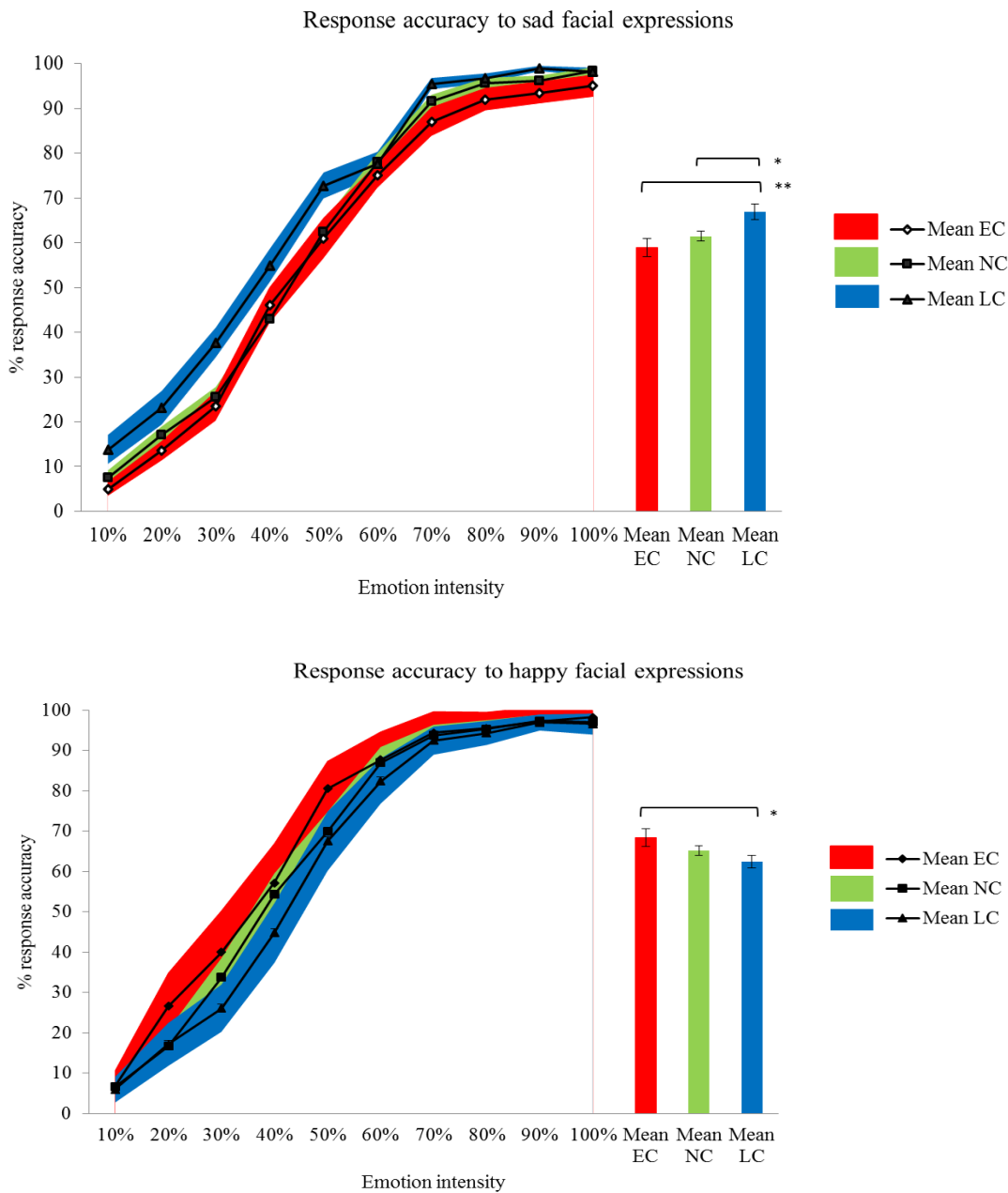


Figure 10. Intensity-accuracy response curves to sad (top figure) and happy (bottom figure) facial expressions for early (EC), neither (NC) and late (LC) chronotype groups (shaded areas shows standard error). With the exception of the extreme points of the curve, LC response accuracy appears to be shifted to the left (relative to NC and EC) indicating this chronotype group are more accurate at recognising sad facial expressions. EC response accuracy appears to be marginally shifted to the left in the happy facial expression graph. Mean response accuracy for all three groups are displayed on the right-hand side of the figures where error bars represent standard error of the mean. (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ ).

In an exploratory analysis, there was a significant difference between chronotype groups in accurately recognising ambiguous happy faces (50% intensity) (one-way ANOVA  $F(2,225) = 3.49$ ,  $p = .032$ ) and ambiguous sad faces (50% intensity) (one-way ANOVA  $F(2,225) = 3.99$ ,  $p = .02$ ) such that LCs ( $M = 72.7$ ,  $SD = 24.1$ ) were more accurate at recognising ambiguous sad faces than EC ( $M = 60$ ,  $SD = 32.1$ ) and NT ( $M = 62.7$ ,  $SD = 26$ ) and less accurate ( $M = 67.5$ ,  $SD = 30.1$ ) at recognising ambiguous happy faces than EC ( $M = 80.5$ ,  $SD = 22.5$ ) but not NC ( $M = 69.9$ ,  $SD = 26.2$ ). There were no differences between chronotype groups in accurately recognising neutral faces (one-way ANOVA  $F(2,225) = 2.59$ ,  $p = .077$ ).

### 3.4. Discussion

The current findings replicate and extend previous evidence that emotional processing biases exist in LC individuals, who are at risk of depression but have never been depressed. LC individuals were found to be more accurate at recognising sad facial expressions than EC and NC individuals, and recognised fewer happy facial expressions than ECs but not NCs. LC were also more accurate at identifying ambiguous sad faces and less accurate at recognising ambiguous happy faces. These differences were present despite all three chronotype groups being similar in terms of sleep quality and depression/anxiety levels, and were not driven by small differences in age and gender between groups or possible (a)synchrony effects. The current data replicate and extend earlier findings that EIC types have reduced accuracy for sad faces (Berdynaj et al., 2016) and increased accuracy for happy faces (Chapter 2) to better demarcate chronotypes that previously were missed due to the collapsing of early/neither chronotypes into a single group. Due to the nature of recruitment in this study, a number of advantages can be noted. Firstly, the sample size was significantly larger than previous studies and included a more diverse range of participants (not limited to university students with a

restricted age range and similar educational backgrounds). Moreover, participants were truly free to complete the experiment at the time of their choosing compared to previous studies that are often limited to allotted time-slots. This therefore adds to the validity of the results.

As discussed in Chapter 2, Chan and colleagues (2007) suggested that decreased processing of positive stimuli in at-risk individuals (such as high neuroticism) may progress to additional increased processing of negative stimuli should depression be triggered (Chan et al., 2007). This hypothesis is consistent with findings reported in Chapter 2 but here, LC individuals recognised significantly more sad faces as well as fewer happy faces. This could be explained by the increased power in this study and more distinct chronotype groups. Also, facial expressions in this online experiment were presented such that participants could spend as little or as much time deciding the emotional valence of the face in comparison to most studies that present stimuli for fixed periods (typically 100 ms – 2000 ms (e.g. 500 ms in Chapter 2)). Surguladze and colleagues (2005) reported that biases towards happy and sad faces were better revealed with different stimulus presentation timings (Surguladze et al., 2005) i.e. conscious vs. automatic processing, suggesting another explanation for the discrepancies between studies. Alternatively, it may be that different emotional processing biases exist in relation to late chronotype and familial risk for depression (Dainer-Best et al., 2018; Taylor & Ingram, 1999) compared to neuroticism.

A converging corpus has identified that late chronotype is associated with an increased prevalence of depression (Hidalgo et al., 2009; Konttinen et al., 2014; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) that appears independent of sleep quality. The results of the current study show that healthy, never-depressed individuals display a pattern of emotional biases similar to those reported in acutely depressed (Bradley et al., 1995; Gilboa-Schechtman, Erhard-Weiss, & Jeczemien, 2002; Gur et al., 1992; Peckham et al., 2010; Ridout, Astell, Reid, Glen, & O'Carroll, 2003; Surguladze et al., 2004) and remitted depressed patients



(Anderson et al., 2011; Bhagwagar et al., 2004; Hayward et al., 2005). Cognitive theories of depression posit that negative schemata constrain how emotional information is attended to, processed and recollected and that this negative emotional bias is important in the aetiology and maintenance of depression (Beck, 1989). Our data, therefore, suggest that certain negative biases (including to facial expressions as reported here) may exist prior to the onset of depression in LC individuals which may reflect a trait vulnerability marker for the disorder.

### 3.4.1. Specific limitations

The nature of the current study (anonymous online survey) required that current, previous and family history for depression and diagnosed sleep disorder was determined by self-report rather than reference to medical history or structured clinical interview. Although this method allowed a large sample size to be obtained, the possibility that some participants may have met these exclusion criteria and contributed to the data presented cannot be fully excluded. Secondly, high neuroticism personality trait was not measured or excluded in participants in this experiment which is another population shown to be at-risk of depression (Kendler, Gatz, et al., 2006). Neuroticism has been related to late chronotype in some studies (DeYoung, Hasher, Djikic, Criger, & Peterson, 2007) but not others (Hogben, Ellis, Archer, & von Schantz, 2007) and was weakly associated with chronotype in Chapter 2. However, biases in emotional processing were reported in similar samples of adults despite no observable differences in neuroticism (Berdynaj et al., 2016) or when neuroticism was controlled for during analyses (Chapter 2) suggesting the current findings were not driven by differences in neuroticism. Finally, this study was limited to measuring only two emotions (happiness and sadness) whereas it is known that depressed patients show biases to a range of emotions e.g. fear, disgust (Sheline et al., 2001). Future studies including a range of emotions are warranted.

### 3.5. Conclusion

In conclusion, late chronotype was associated with increased processing of sad faces and decreased processing of happy emotional facial expressions. These findings suggest late chronotypes have emotional processing biases that are similar to those seen in individuals with depression and may explain why this group are more at risk of developing the disorder. Future studies exploring facial expression recognition using a wider range of emotions are needed.

## **CHAPTER FOUR**

### **Introduction to Magnetic Resonance Imaging**

## 4.1. General introduction

As well as the cognitive and behavioural changes that are observed in patients with depression (discussed in Chapter 1), there are also a number of neural processes that are impaired, including structural and functional differences. The main aim of the following chapters will be to investigate whether late chronotypes have similar structural and functional differences to patients with depression using Magnetic Resonance Imaging (MRI) which could represent vulnerability for the disorder. This second introductory chapter will therefore introduce MRI imaging including functional MRI (fMRI), structural MRI (sMRI), resting-state functional MRI (rsfMRI) and magnetic resonance spectroscopy (MRS). This will be followed by an outline of the neural systems that can be measured using these methodologies including; emotion processing, brain structure, resting-state networks and neurotransmission (respectively), followed by a review of the neural mechanisms that are impaired in major depression.

## 4.2. Magnetic Resonance Imaging (MRI)

The past 20 years have given rise to significant developments in MR imaging. This technique is a safe, non-invasive method of producing high resolution images of soft tissues such as the brain and demonstrating dynamic physiological changes representing neural activity. The MR system is comprised of a number of components, including; a large electromagnet that generates a static magnetic field ( $B_0$ ), shim coils to ensure the magnetic field is homogenous, a radiofrequency (RF) coil to transmit RF pulses, a receiver coil to detect the

MR signal, gradient coils to encode the spatial localisation of the signal, and a computer to reconstruct the signal into an MR image.

MRI is based on the principle of nuclear magnetic resonance (NMR) which is founded on the existence of *spin* in many subatomic particles, including hydrogen which is abundant in the human body (Bloch, 1946; Purcell, Torrey, & Pound, 1946). The spin of the hydrogen proton gives it a magnetic property, and angular momentum, that when placed in an applied magnetic field ( $B_0$ ), it tends to align with the field in a low energy state and wobble or “precess” about the direction of the magnetic field. The proton’s precession frequency (or Larmor frequency ( $\omega$ )) depends on the magnetic field strength (commonly 3 Tesla) (Plewes & Kucharczyk, 2012). By exposing the hydrogen protons to an alternating magnetic field ( $B_1$ ) and applying RF pulses, a proportion of protons are tipped into the transverse plane where they induce a signal in the receiver coil. The time taken for the proton’s longitudinal magnetisation to then return to 63% of its final value is referred to as  $T_1$  relaxation. In addition to  $T_1$  relaxation, subtle differences in the magnetic field cause the protons to interact and modify their precession rate and become out of phase in the transverse plane. The time taken for the protons to return to 63% of its original transverse magnetisation is referred to as  $T_2$  relaxation.  $T_1$  and  $T_2$  relaxation times of different tissues, e.g. grey matter, white matter and cerebro-spinal fluid (CSF), have been catalogued and are used as key determinates of contrast as well as to detect pathologies such as neoplasms (Bottomley, Hardy, Argersinger, & Allenmoore, 1987). By measuring the  $T_1$  and  $T_2$  relaxation times of the protons, the type of tissue the proton is in can be determined.

In order to identify the location of the proton signal in the brain, magnetic field gradients (a non-uniform magnetic field) are applied to encode the location of the proton depending on its Larmor frequency. The gradient pulses applied to the brain allow the data to be collected in slices (slice-select gradient) and then each slice is arranged in a matrix (including rows and

columns) of individual tissue voxels. The slices are excited with frequency-encoded gradients and phase-encoded gradients so that the position of the individual voxels can then be determined by their frequency and phase. To produce the final MR image, the data must go through a signal acquisition phase and an image reconstruction phase. During signal acquisition, the Fourier transform, invented by Jean Baptiste Joseph Fourier, is used as a mathematical way to transform amplitude, density, angle and phase from the MR signals into an array of stripe patterns (sine and cosine waves) that are represented in a temporary image space. The temporary image space is known as *k space* which has coordinates that define its spatial frequency in the X and Y directions. At the end of the scan, the inverse Fourier transform is then used to sample *k space* and reconstruct the original image (Plewes & Kucharczyk, 2012).

#### 4.2.1. Pulse sequences

The contrast of the image is dependent on the pulse sequence used. A pulse sequence is a set of RF and gradient pulses delivered to the sample tissue which can differ in number, strength and timing. The timing of these pulses is the most important parameter: the repetition time ( $T_R$ ) is the time between consecutive RF pulses, and the echo time ( $T_E$ ) is the time between the initial RF pulse and the echo (peak of the signal induced in the coil). To produce  $T_1$ -weighted images, a short  $T_R$  (<1000 ms) and a short  $T_E$  (<30 ms) are used because the  $T_1$  decay of water is much slower and so structures containing a high water content (e.g. the CSF) appear much darker on the image. In research, a  $T_1$ -weighted image is usually collected at the beginning of the scan in order to register the functional MRI data (typically collected with relatively low resolution) onto a high resolution anatomical image. To produce  $T_2$ -weighted images, a long  $T_R$  (> 2000 ms) and long  $T_E$  (> 80 ms) are used which makes the CSF appear bright.  $T_2$ -weighted images, therefore, are particularly useful for detecting brain pathologies such as oedema which have a high water content. Many other pulse sequences exist which

optimise the tissue contrast depending on the structure of interest, most recently the Fluid Attenuated Inversion Recovery (FLAIR) sequence produces  $T_2$ -weighted images with a suppressed CSF signal.

#### 4.2.2. Resolution of the MR image

Spatial resolution of an MR image is determined by the size of the imaging voxels (3D volumes of data that make up the image). The smaller the size of the voxel, the higher the spatial resolution and the more ‘detailed’ the image is which is important for localising brain structures. The size of the voxels depend on the matrix size (number of frequency-encoding and phase-encoding steps), field of view (FOV) (size of area the matrix covers) and slice thickness. By increasing the matrix size, or decreasing the FOV and slice thickness, this will increase the spatial resolution of the image. However, this is at the expense of the signal-to-noise ratio (SNR): a measure of signal strength which is essential to obtain good quality data. To improve the SNR, the  $T_R$ , FOV and slice thickness can be increased or the  $T_E$  and matrix size can be decreased. To obtain images with high spatial resolution and good SNR requires longer scan times which is unfavourable for safety reasons (recommendations vary but for studies at Roehampton participants should not be in the scanner longer than 1.5 hours) and limits the number of scans that can be performed in a single session (Amaro & Barker, 2006). Therefore, the scanning parameters must be optimised for the type of image required; for example, the FOV is often restricted to a smaller section of the brain that covers a structure of interest, e.g. the amygdala. During fMRI, the temporal resolution must also be accounted for – a shorter  $T_R$  will allow the time series of a neural event to be better resolved but this is at the expense of spatial resolution (except at high magnetic fields e.g. 7T) or reduced FOV. Typical scanning parameters for fMRI echo planar imaging are:  $T_R = 2000$  ms,  $T_E = 30$  ms,  $FOV = 192 \times 192$

mm, 64 x 64 acquisition matrix, 3 x 3 x 3 mm<sup>3</sup> voxels, 30 slices. However, the recently developed multiband technique is becoming increasingly popular in fMRI research. This technique uses RF pulses that excite multiple slices at the same time which means the acquisition time can be significantly reduced (depending on the multiband factor) without shortening the T<sub>E</sub> or sacrificing SNR (Feinberg & Setsompop, 2013).

### 4.3. Functional MRI

Functional MRI (fMRI) is the hugely popular imaging technique based on MRI that was developed to indirectly detect regional changes in brain metabolism over time. MR images are therefore collected every 2-3 seconds (depending on the paradigm). The imaging technique is based on the evidence that neurotransmission in the brain requires energy in the form of adenosine triphosphate (ATP), and increased neural activation in a specific brain region triggers the haemodynamic response, which can be measured using the Blood Oxygen Level Dependent (BOLD) signal.

#### 4.3.1. The BOLD response

Functional MRI uses the BOLD response to a task as an indirect measure of neural activity. Neural activation in response to a specific task, e.g. visual checkerboard stimulation, results in an increased energy demand and an increased cerebral metabolic rate of oxygen (CMRO<sub>2</sub>) in the associated brain region (Buxton & Frank, 1997). There is therefore an initial drop in local oxygenated haemoglobin and increase in deoxygenated haemoglobin. This causes the waste products of glycolysis to build up and trigger chemical signals such as nitric oxide (NO) to activate vasodilation in the local capillary bed. Following a lag of 2-6 seconds, cerebral

blood flow (CBF) increases and delivers a surplus of oxygen to the affected region resulting in a change in the ratio of oxygenated/deoxygenated haemoglobin i.e. an increase in oxygenated haemoglobin and decrease in deoxygenated haemoglobin. Functional MRI therefore uses BOLD contrast to detect changes in brain metabolism representing neural activity by imaging changes in the ratio of oxygenated and deoxygenated haemoglobin (Ogawa, Lee, Kay, & Tank, 1990). This is because deoxygenated haemoglobin is paramagnetic (will interact with the local magnetic field) and causes local dephasing of protons and a reduced signal, whereas oxygenated haemoglobin is diamagnetic and will not. The increase in neural activity will produce an increase in the oxy/deoxyhaemoglobin ratio leading to an increased MR signal proximal to the area of increased oxygen metabolism. Pulse sequences designed to be  $T_2^*$ -weighted (using gradient echos with relatively long  $T_E$  values to accentuate local magnetic homogeneity effects) are specialised to be sensitive to this change in ratio, which is about 1-5%. The change in BOLD signal is proportional to the underlying neural activity and will eventually plateau if the stimulus is sustained for a long period, or return to baseline if the stimulus is removed. The signal intensity of each voxel collected during acquisition is then compared to a model of the expected BOLD response to the stimulus, and statistical tests are used to detect (small) significant signal changes which represent changes in neural activity.

#### 4.3.2. Experimental design

It is important to note that the BOLD response is not a quantitative measure of neural activity and unlike other neuroimaging techniques, such as arterial spin labelling, the units of measurement are arbitrary. Functional MRI studies are designed to induce different neural states using various stimuli which are then compared against each other, or against a 'control' condition e.g. fixation cross. It is therefore important to consider the experimental design used in the study to ensure the different neural states can be interpreted and compared effectively



after data acquisition. The two main types of fMRI experimental design are the block design and event-related design. Using a block design, trials are arranged in ‘blocks’ and alternate between experimental and control conditions (e.g. ABACAB...) usually lasting around 20 seconds each. The data is then averaged across blocks for each participant and conditions are compared by ‘subtracting’ one from the other to identify brain regions representing performance of the task. This design has good power to detect voxel activation, localise functional areas and study steady-state processes. Alternatively, an event-related design presents stimuli briefly (a few seconds) in non-constant intervals. This design is better for detecting transient changes in brain activity for each individual (e.g. errors) and preventing the participant from predicting stimulus presentations or habituating to the task. However the design also lacks power because fewer events are averaged.

#### 4.3.3. fMRI analysis

Once the images have been collected, the data needs to be pre-processed in order to remove artifacts, meet key statistical assumptions for analyses and standardise the location of brain regions across subjects. This is applied to both fMRI and structural images and a number of software packages (e.g. FSL or SPM) offer a selection of pre-processing and analysis tools. Pre-processing steps typically include: 1) non-brain removal to remove tissue and skull outside of the brain 2) slice-timing correction to adjust for differences in the timings of each slice acquisition, 3) rigid-body realignment to minimise the effect of head movement within the scanner by realigning images to a common reference image (typically the first, the middle or a mean image) using 6 translation and rotation parameters and then re-slicing them to represent the original data. Physiological noise (e.g. cardiac and respiratory cycles) can also be recorded via bellows and pulse oximeters and regressed from the overall model, 4) co-registration of the fMRI images onto a high resolution structural image using an affine transformation with 12

degrees of freedom, 5) temporal filtering generally using a high-pass filter to remove low frequency noise such as scanner drift (Lindquist, 2008), 6) distortion correction using field maps acquired in the same session as the functional data to correct for distortions that arise due to homogeneities in the  $B_0$  field, especially around air-tissue interfaces, 7) spatial normalisation of structural and fMRI images from multiple participants into a common space (e.g. Montreal Neurological Institute (MNI) atlas space), and 8) smoothing (spatial filtering) where the image is convoluted with a 3D Gaussian filter (kernel) measured at the full width at half maximum (FWHM)(usually twice the dimensions of the voxels) to improve the SNR and reduce anatomical differences between participants. A number of quality control procedures should also be performed during pre-processing. For example, investigating the absolute and relative motion parameters for each subject after motion correction and excluding participants displaying large head movements. Additional artifact removal can also be applied using independent component analysis (ICA) to identify independent components in the data and remove those that are characterised by noise or head movement.

#### 4.3.4. Statistical modelling

After pre-processing, the data can be analysed to localise brain activity, firstly at the individual subject level (first-level analysis) and then at the group level (second-level analysis). The most common first-level analysis method for modelling the activity in each voxel is the General Linear Model (GLM). This approach models the relationship between one or more explanatory variables and the response variable (BOLD signal) by fitting a linear equation to the observed data. In its most basic form, the linear equation model is;  $Y = X\beta + \epsilon$ , where  $Y$  is the BOLD signal time series.  $X$  represents the design matrix constructed using the predictor variable(s) i.e. the stimulus function (from the paradigm) convolved with the canonical HRF (e.g. double gamma function) to produce the predicted BOLD signal for each experimental

condition(s). Temporal derivatives and nuisance regressors (e.g. estimated motion parameters) may also be included in the model to increase statistical sensitivity.  $\beta$  represents the unknown set of weights (magnitude and direction) between the predictor variables and BOLD signal that need to be estimated, and  $\epsilon$  is the error value. The GLM model estimates the  $\beta$  values for each voxel using the ordinary least squares solution (minimising the sums of squared errors). A t-test (or F-test, depending on the design) is then used to test if  $\beta$  (the effect of X on Y) is significantly different from 0 in each voxel using a mass univariate approach (voxel-wise analysis). A statistical map of significant neural activations is produced which can be overlaid onto a structural MR image. Tests can also be performed to investigate task-related activation depending on pre-determined contrasts of interest.

At the group analysis stage, a mixed effects (or random effects) model is most commonly used to estimate the fixed effect  $\beta$  coefficient (from the single-subject analysis) and the variance components between-subjects. These parameter estimates are then used in the statistical design to test whether there are significant activations on average across the sample in relation to the contrast of interest (e.g. fearful vs. happy faces) or if these are associated with a covariate e.g. chronotype (which must be mean-centred). Covariates of no interest are also often included in the model in order to rule out the effect of confounding factors e.g. age. The statistical inference can be performed using parametric or non-parametric approaches. Voxel-wise inference is a parametric approach that treats each voxel independently and performs the statistical test (e.g. one-sample t-test) at each individual level i.e. testing whether each voxel intensity exceeds a threshold of significance. However, cluster-based statistics is a more robust approach to use as it considers the spatial extent of the voxel activation rather than just the peak height, by treating voxels as contiguous (related). Clusters are first defined using a threshold (usually  $z = 3.1$ ) and then cluster significance is tested by comparing the size of each cluster to a critical cluster size threshold. Both statistical approaches require correction for the multiple

testing problem which occurs when multiple tests are performed, increasing the chance of false positives (especially in the case of fMRI when thousands of data points are being tested). Random Field Theory is commonly used to account for the multiple comparison problem by correcting the autocorrelated, ‘smoothed’ data to the family-wise error rate (corrected p-value of 0.05).

#### 4.3.5. Permutation testing

Permutation testing is a non-parametric approach to statistical inference. This approach is more commonly used now as a result of recent research that suggests conventional parametric methods (such as the voxel-wise and cluster-based approaches described above) are not robust because these tests assume that voxel activations are independent, that the underlying smoothness of the image is constant across the entire brain and that fMRI data is normally distributed which is generally incorrect (Eklund, Nichols, & Knutsson, 2016). Functional MRI studies also often suffer from small sample sizes and there may be concerns with assumptions of normality. Permutation testing does not assume the data are normally distributed. Instead, the method ‘shuffles’ the data (applying 5000 permutations as standard) to determine the exact distribution and then compares this to the observed data to test whether it is significantly different using a corrected p-value of 0.05. Threshold-free cluster enhancement is often used as well which allows cluster correction to be performed without requiring an arbitrary threshold. Permutation testing will be used consistently in the following neuroimaging chapters.

#### 4.3.6. Region of Interest

After whole-brain analyses are executed, further analyses can be performed in order to investigate a particular region of interest (ROI). ROI analysis may be done for a number of reasons, including; 1) to control for Type 1 errors, 2) to explore the data, or 3) test a region that

has been previously functionally defined (Poldrack, 2007). The first approach is commonly used to specify one or more anatomical ROIs *a priori* in order to perform statistics across these regions and reduce the number of stringent corrections for multiple tests necessary for whole-brain analyses (small volume correction). For example, the *a priori* ROI in the following chapter is the amygdala because this region has been previously shown to be robustly activated in response to an emotional stimulus. The ROI statistical map is thresholded and then the number of activated voxels is usually reported. Using the second approach, an exploratory ROI analysis is usually performed by creating small ROIs (e.g. spheres) at the peaks of activation clusters or at local maxima within a cluster, and masking the ROI with a thresholded activation map to identify regions that are activated across conditions. Thirdly, an ROI can be performed using a functional localiser in order to examine its sensitivity with something else (e.g. connectivity with other brain regions). Again, these ROI analyses can be performed using non-parametric statistics including permutation testing. In either case, clear justification for any ROI analyses must be presented. For example, defining an ROI based upon a significant activation during whole-brain analysis can be useful for exploring patterns of activation in complex models, but should not be used for statistical inference because the analyses are not independent and are therefore substantially biased.

## 4.4. Structural MRI

In contrast to fMRI, structural MRI uses an individual image of the brain (T<sub>1</sub>-weighted image) to explore differences in structure across participants. Structural MRI data is commonly analysed using voxel-based morphometry (VBM) within FSL or SPM. The raw data are first pre-processed where structural images are brain-extracted and grey matter-segmented before being registered to a standard space using non-linear registration (Andersson & Smith, 2007).

The resulting images are averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images are non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images are then smoothed with an isotropic Gaussian kernel similar to functional data. The images are then segmented into their three tissue compartments; grey matter, white matter and cerebrospinal fluid (CSF) based on the intensity of the voxel using a mass univariate approach with multiple comparison correction (Martin, Bender, & Focke, 2015). A partial volume probability map is produced assigning each voxel with a certain tissue class using the hidden Markov random field (HMRF) model (Zhang, Brady, & Smith, 2001). The actual tissue volume ( $\text{mm}^3$ ) can then be calculated by multiplying the mean value (from the partial volume map) with the brain structure volume (in voxels). Alternatively, a ROI approach can be used by defining the structure of interest with an atlas toolbox or by manually tracing structures by hand. Volume differences can then be compared between-subjects using appropriate statistical tests.

Additionally, structural differences in particular brain regions of interest can be compared across subjects by segmenting subcortical structures. The segmentation procedure can be done manually or more recently using automated procedures in FSL or FreeSurfer. Manual segmentation requires tracing the brain region by hand onto high resolution MR images. However, this is a labour intensive process lasting long periods of time and relies on subjective interpretations of anatomical variations, so it is most consistent when a single expert in neuroanatomy traces the whole dataset. Automated methods have therefore become more popular and provide roughly similar reproducibility values and estimates of volume change depending on the structure of interest (Mulder et al., 2014). FSL's FIRST segmentation uses a Bayesian probabilistic approach and is based on shape/appearance models constructed from 336 manually segmented images provided by the Centre for Morphometric Analysis (CMA),

Boston (Patenaude, Smith, Kennedy, & Jenkinson, 2011). The manual labels are parameterised as surface meshes which represent volumetric information for each structure. The most probable shape is then ascertained by searching through linear combinations of shape modes of variation based on learned models. The segmentation process also includes boundary correction and registration to a standard MNI152 template at 1mm resolution using 12 degrees of freedom, and to a subcortical mask in order to eliminate voxels outside the subcortical structure. The volume of the structure can then be computed and compared across groups using appropriate statistics.

Finally, structural MRI data can also be analysed using shape analysis to investigate localised morphological differences in a structure of interest. This can be done using automated software including surface-based vertex analysis in FSL which is also more sensitive to volume differences in subcortical structures than VBM analysis. Again, this analysis is based on multivariate statistics which creates a 3D mesh of the structure of interest after it has been segmented (using the methods above) and compares the shape of the structure (i.e. the position of the vertices) across groups. Alternatively, structures such as the hippocampus can be manually segmented into anterior (head) and posterior (body and tail) regions and their volumes calculated separately and compared across participants.

## 4.5. Resting-state fMRI

Resting-state fMRI (rsfMRI) is a common FMRI method where Blood Oxygenation-Level Dependent (BOLD) data is acquired to evaluate functional connectivity while participants are at rest (not performing any specific task) and are given minimal instruction (e.g. keep their eyes open; let their mind wander). A number of techniques have been developed to probe rsfMRI data. In model-free approaches such as Independent Components Analysis (ICA),

the spatio-temporal structure of the data is characterised into a number of independent components reflecting a functional network, physiological noise or image/acquisition artifact. Further processing is then required to either manually select relevant networks and discard noise components or use a well validated resting-state network map (Smith et al., 2009), to generate subject-wise networks for subsequent analysis (Filippini et al., 2009). These spatial maps can then be processed using dual regression. Firstly, spatial regression is used to determine the time series associated with the voxels in the spatial maps. Then, temporal regression uses the time series (identified using spatial regression) to find the full set of associated voxels specific to each subject. The spatial maps can then be compared between subjects using permutation testing. An advantage of using this approach to measuring resting-state connectivity is that it is not based on spatial assumptions and pre-existing networks. It therefore lends itself to identifying unexpected areas of interest. However, defining the optimal number of components to be generated is arbitrary and varies from study to study which effects the number of patterns of connectivity that can be derived.

Other analytical approaches include seed-based correlation analyses - where the time-course from a 'seed' voxel (region of interest) is extracted and correlated with activity at each voxel (van den Heuvel & Hulshoff Pol, 2010). This approach allows for a much more straightforward interpretation of the data but limits the findings to networks that display connectivity only with the region of interest. Less commonly used techniques include regional and network homogeneity which assess, respectively, the correlation between a given voxel and its nearest neighbours or all voxels in the entire network. Finally, Amplitude of Low Frequency Fluctuations (ALFF) and fractional ALFF (fALFF) quantify the amplitude of low frequency oscillations generated in the brain at rest.



## 4.6. Magnetic Resonance Spectroscopy

Proton magnetic resonance spectroscopy (MRS) is a non-invasive analytical technique associated with MRI imaging. Much like fMRI, MRS exploits the magnetic properties of atomic nuclei in the brain that possess spin such as hydrogen ( $^1\text{H}$ -MRS). Instead of causing the spins to transition from an antiparallel to parallel state as with many fMRI sequences, spectroscopy sequences cause the spins to become polarised with the RF field and rotate along the z-axis of the magnet. This creates a rotating magnetic field at the Larmor frequency which induces an oscillating voltage that is detected by the MR spectrometer (RF receiver) (Lei, Xin, Gruetter, & Mlynárik, 2014). The hydrogen spins have small differences in frequency depending on the molecular structure the atomic nucleus is within and the chemical environment and geometric composition of the molecule, or 'J-coupling'. Specific nuclei contained within a metabolite therefore give rise to either a single peak or multiple peaks that are uniquely positioned along the frequency axis of the spectrum (Richards, 2001). This is also known as the 'chemical shift'. The relative concentrations of metabolites are then calculated from the areas under the spectrum peaks. These include aspartate, gamma-amino butyric acid (GABA), glucose, glutamate (Glu), glutamine (Gln), lactate, N-acetyl aspartate (NAA) and many more that have critical functions in the brain including neuroenergetics, neurotransmission, and neuromodulation. MRS therefore provides information about the biochemical composition of the brain as well as many pathophysiological processes including tumours (Gujar, Maheshwari, Bjorkman-Burtscher, & Sundgren, 2005).

The most common method of MRS is single voxel spectroscopy (SVS) which samples spectra from a single, predefined voxel (Lei et al., 2014). In research, it is commonly used to probe the hydrogen nucleus ( $^1\text{H}$ -MRS) whereas the carbon nucleus ( $^{13}\text{C}$ -MRS) is often used clinically to assess disorders of brain metabolism. A voxel of interest, usually measuring 30 mm x 20 mm x 20 mm is placed in a pre-selected anatomical, functional or clinical target area

of the brain in order to collect spectra from. This has included, but is not limited to, the anterior cingulate cortex (ACC), dorso-lateral prefrontal cortex (DLPFC) and amygdala as well as the occipital cortex which is used to provide a global measure of brain chemistry. Specialised MR pulse sequences are used to acquire MRS data including; Stimulated Echo Acquisition Mode (STEAM), Point REsolved SpectroScopy (PRESS), and more recently, SPin ECho full Intensity Acquired Localised (SPECIAL).

MRS is not a very sensitive technique and so a number of procedures are performed to enhance the signal. MRS data is acquired by suppressing the water signal in the spectrum and by performing outer volume suppression around the outside of the volume of interest to minimise the signal from other brain regions. Shimming is also applied to ensure a homogeneous magnetic field needed to enhance the sensitivity and resolution of the acquired spectra by narrowing the peak width, increasing the SNR and improving water suppression. High field strength is important to gain adequate SNR for spectroscopy and is usually only performed at 3T or higher. Despite these processes, some regions are hard to study using MRS including subcortical structures (e.g. amygdala) that are near to air-tissue interfaces that cause regional inhomogeneities in the magnetic field and contaminate the spectra. It is therefore important to ensure the quality of the spectra is good and often spectra are excluded if they do not meet quality control criteria (usually Cramer-Rao Lower Bound (CRLB) < 20%). Additionally, some neurometabolites are difficult to measure at low field strengths (1.5T or lower) or with certain pulse sequences that are not optimised to detect them. For example, overlapping glutamatergic signals (glutamate, glutamine and glutathione) are not easily separated and so a composite measure of these signals (Glx) is sometimes used as a surrogate marker for glutamate.

MRS data can be processed using a number of software programs including LCModel (Provencher, 1993) and TARQUIN (Wilson, Reynolds, Kauppinen, Arvanitis, & Peet, 2011).

First, the data goes through a set of pre-processing steps that can include; 1) zerofilling to improve spectral visualisation, 2) windowing and filtering to improve the SNR, or the spectral resolution, or to remove artifacts or broad spectral components, and 3) correction for eddy current artifacts and frequency shifts using an unsuppressed water signal reference. Following spectral processing, the spectra are averaged across the total scan time and analysed for a range of metabolites by fitting a chosen metabolite basis set which contains a standardised set of metabolite profiles. The absolute concentrations of metabolites can be measured by referencing them to either the tissue water signal (absolute concentrations) or to total creatine (creatine plus phosphocreatine). Both references have been widely used although both methods have their disadvantages; the water peak can be affected by oedema but levels of total creatine can change during early development and in brain tumours (Minati, Aquino, Bruzzone, & Erbetta, 2010). More generally, MRS has poor sensitivity and so cannot be used to detect molecules that are present in the brain in lower quantities such as serotonin. It also cannot provide specific information about where the neurometabolite is in the voxel of interest (e.g. in neurones or astrocytes).

In summary, MRI imaging can be used in many different forms to investigate different structural and functional aspects of the brain. The technique relies on careful data collection and stringent analysis and statistical procedures to inform us of the underlying neural systems and processes it measures. The next section of this introductory chapter will therefore provide an outline of the neural systems that can be measured using these MRI techniques, followed by a review of the neural mechanisms that are impaired in major depression.

## 4.7. Neural substrates of emotional processing using fMRI

In addition to the cognitive research into emotional processing, the underlying neural mechanisms of emotional processing have been investigated using neuropsychological approaches including fMRI imaging. As outlined by Phillips and colleagues (2003a), processing of an emotive stimulus is thought to require 1) identification of its emotional significance, 2) production of a response, including autonomic, neuroendocrine and behavioural responses leading to an affective state, and 3) regulation of the emotional response which could involve inhibition or modulation (Phillips, Drevets, Rauch, & Lane, 2003a). The authors further implicated specific brain regions responsible for these processes into a ventral and dorsal neural system. Overall, the ventral system, including the amygdala, insula, ventral striatum and ventral regions of the ACC and PFC, is responsible for evaluating the emotional significance of the stimulus and producing the affective state as well as automatically regulating the response to the emotive stimulus. Whereas, the dorsal system, including the hippocampus and dorsal regions of the ACC and PFC is important for integrating cognitive processes and executive function (which can be biased by emotional input) as well as effortful regulation of the affective state. These two neural systems are thought to have a reciprocal functional relationship (Phillips, Drevets, Rauch, & Lane, 2003b).

A wide range of experimental paradigms have been used to investigate the neural mechanisms responsible for emotion processing. In particular, experimental paradigms using emotional facial expressions have been used in an attempt to map the neural substrates to realistic stimuli (faces). Emotional reactivity paradigms include attentional deployment-concentration tasks, attention deployment-distraction tasks and cognitive change tasks. Attentional deployment-concentration tasks involve attending to a specific emotion and either responding to the magnitude of the emotion, rating the emotion or monitoring a particular emotion. Attentional deployment-distraction tasks involve attending to something other than

the emotional expression (e.g. gender discrimination) or that the faces are masked i.e. passive viewing of emotional expressions. Finally cognitive change tasks involve instructing participants to consciously change their reaction to an emotional expression (e.g. down regulation). A range of stimuli valences have been used including; fear, disgust, surprise, happiness and sadness. Similarly, other paradigms have been used to explore emotional processes that employ other visual or audio stimuli e.g. emotive words, pictures or scenes, or recordings of negative words and criticisms. Other paradigms are variants of classical cognitive processing and behavioural inhibition paradigms where the stimuli have been replaced with emotionally charged words or images (e.g. go/no-go task, emotional Stroop task). These paradigms have illustrated brain regions used for different aspects of emotional processing.

Taken together, processing emotional information recruits a distinct network of brain regions for specific functions. Abnormalities in this network are thought to underlie differences in emotional behaviour resulting in mood disturbances.

#### 4.7.1. Emotion processing in depression using fMRI

As discussed in Chapter 1, negative biases in emotion processing have been reported in depression. In parallel to this, impaired functional neural substrates responsible for emotion processing and regulation have been identified in depression using fMRI. For example, functional neuroimaging studies of depression have consistently reported increased amygdala reactivity to negative emotional stimuli, including facial expressions (Disner, Beevers, Haigh, & Beck, 2011). In response to negative stimuli, depressed patients typically show a more intense and sustained amygdala response indicative of biased stimulus processing. Indeed, many previous studies report that amygdala, as well as parahippocampal gyrus, activity is an indicator of the emotional intensity experienced by an individual. This pattern of elevated

amygdala response is automatic (i.e. is present even when the stimuli are presented subliminally and the participant is unable to verbally label the valence of a stimulus) and can be reversed with pharmacotherapy (Anand, Li, Wang, Gardner, & Lowe, 2007; Fu et al., 2004; Sheline et al., 2001).

According to a review by Disner and colleagues in 2011, functional impairments in the emotional processing circuitry correspond to five cognitive biases associated with the symptoms of depression; 1) biased attention, 2) biased processing of emotional stimuli, 3) biased thoughts and rumination, 4) biased memory for negative stimuli, and 5) dysfunctional attitudes and negative schemas (Disner et al., 2011). It is suggested that the increased amygdala activity to negative facial expressions may result in a restricted range of emotions and symptoms of depressed mood and anhedonia because of the predominant perception of negative emotions (fear, anger, sadness, disgust) rather than positive emotions (happiness) by the amygdala (Phillips, Drevets, Rauch, & Lane, 2003c). Moreover, unconscious processing of facial expressions requires assessment of the affective content, significance and salience of the faces which has been hypothesised to be mediated through the amygdala (with a lateralisation to the right amygdala) and its direct feedback mechanisms (Morris, Ohman, & Dolan, 1998; Vuilleumier, 2005). The enhanced amygdala activity observed in depression may therefore represent a process of assigning more salience and attention to the negative facial expressions and fuelling negative thinking processes including biased thoughts, rumination, biased memory recall, dysfunctional attitudes and negative schemas. Indeed, differences in activity in extra striate regions (e.g. fusiform gyrus and cuneus) responsible for processing faces are present in depression. For example, a reduction in neural response to happy facial expressions is observed whilst an increased response to negative facial expressions is associated with depression (Keedwell et al., 2005; Lawrence et al., 2004; Surguladze et al., 2005). Taken together, these

automatic biases in emotional processing by the amygdala may be important for the aetiology and maintenance of depression.

A number of functional impairments have also been observed in brain regions responsible for top-down inhibition of the amygdala. For example, increased activation in the left orbitofrontal, left superior frontal and anterior cingulate gyrus was observed during suppression of negative emotions compared to maintaining negative emotions (Phan et al., 2005), and this enhanced activity was related to a reduction in self-reported negative effect (Mak, Hu, Zhang, Xiao, & Lee, 2009). In major depression, however, frontal cortical regions show abnormal activity. For example, the dorsolateral prefrontal cortex (DLPFC) shows hypoactivation in response to emotional stimuli and reduced inhibition of the amygdala. Hypoactivation of the DLPFC as well as the ventrolateral PFC (VLPFC) and superior parietal cortex is related to an inability to block out negative thoughts (symptoms of negative rumination) and difficulty disengaging from negative stimuli i.e. attentional bias towards negative stimuli (Beevers, Clasen, Stice, & Schnyer, 2010). Moreover, hyperactivity in the subgenual anterior cingulate cortex (ACC) (Mayberg, 2003) generally considered the 'affect subdivision', but hypoactivity in the dorsal ACC (Davidson, Pizzagalli, Nitschke, & Putnam, 2002) the 'cognitive subdivision' has been observed in depression. The difference in activity relates to the task that is undertaken; for example, the affect subdivision is generally involved in assigning emotional valence to stimuli, emotional expression and regulating visceral and autonomic responses. Whereas, the cognitive subdivision is involved with processing cognitively demanding information and adjusting emotional reactivity (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Kerns et al., 2004; Pizzagalli, 2011). Taken together, these data suggest that current depression is associated with elevated and sustained neural (amygdala) responses to negative stimuli and impaired emotional regulation associated with an aberrant neural response in higher cortical regions including the ACC. This unregulated amygdala

response generates a ‘bottom-up’ signal that biases emotional processing in higher cortical areas and results in maladaptive perceptions of the environment and social interactions (Disner et al., 2011).

In order to further explore the neural basis of emotional regulation, the functional connectivity between limbic regions and higher cortical areas has been investigated using psychophysiological interaction (PPI) analysis (Friston et al., 1997). The PPI term, which can be added to any linear model, represents the element by element multiplication (interaction) between an input variable (task time course) and a response variable (seed time course). A significant PPI indicates that the correlation in activity between two brain regions is different in different psychological contexts (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). In a meta-analysis of 49 PPI analysis studies, increased functional connectivity with the amygdala was observed in the inferior frontal gyrus, ACC and medial frontal gyrus in a reappraisal vs. maintain condition of an emotional regulation task (Di, Huang, & Biswal, 2017). This pattern of connectivity suggests the higher cognitive areas are effectively down-regulating, or inhibiting, the amygdala response to negative emotion although the direction of this effect cannot be determined. In depressed patients, however, this fronto-limbic connectivity appears to be reduced. For example, Dannlowski and colleagues reported reduced functional connectivity between dACC and amygdala in depressed patients viewing negative (angry and sad) facial expressions compared to neutral expressions (Dannlowski et al., 2009). Moreover, Erk and colleagues reported significantly reduced functional connectivity between left amygdala and right dorsolateral prefrontal cortex (DLPFC) in depressed patients compared to healthy controls (Erk et al., 2010). Hence, there is evidence that neural emotional regulation processes are diminished in depression.

Taken together, these studies have highlighted multiple functional neural abnormalities in depressed individuals including: an elevated and sustained neural (amygdala) response to



negative stimuli, and impaired emotional regulation associated with an aberrant neural response in higher cortical regions including the ACC. These abnormalities may underlie the aetiology and maintenance of the disorder.

## 4.8. Brain structure and grey matter density

As well as exploring which brain regions are functionally activated in response to a task (such as emotional processing), the underlying integrity or density of these brain structures is important to measure in order to facilitate these functions. Overall, brain tissue is made up of neuronal cell bodies (grey matter) and their axons (white matter) which project to different areas of the brain in tracts that form anatomical connections. The grey matter density in a particular brain region is known to positively correlate with various abilities and skills, for example; decreases in grey matter density in the medial temporal lobes in patients with mild cognitive impairment (MCI) correlated with poorer performance on a verbal recall working memory task (Schmidt-Wilcke, Poljansky, Hierlmeier, Hausner, & Ibach, 2009).

### 4.8.1. Structural differences in depression using sMRI

A number of abnormalities in brain structures have been associated with, and may be specific to, major depression. For example, a meta-analysis of 143 studies using MRI reported several brain regions that differ in size compared to healthy controls, including; increased lateral ventricle and CSF volume, reduced volume of the basal ganglia, thalamus, hippocampus, frontal lobe, orbitofrontal cortex and gyrus rectus, and increased likelihood of periventricular and subcortical grey matter hyperintensities (demyelination and axonal loss) (Kempton, Salvador, Munafò, & et al., 2011). In particular, evidence has revealed abnormalities in

structures implicated in emotion and cognition in depression. For example, a meta-analysis of 64 MRI studies of depression reported deficits in PFC, orbitofrontal cortex and the ACC (Koolschijn, van Haren, Lensvelt-Mulders, Pol, & Kahn, 2009). Post-mortem studies of patients with depression have also reported cell loss and atrophy in subgenual prefrontal and orbitofrontal cortex (Rajkowska, 2000). Finally, meta-analyses have also reported volume reductions in the striatum including the putamen and caudate nucleus, structures involved with reward processes and motivation (Koolschijn et al., 2009; Videbech & Ravnkilde, 2004).

Of these brain regions, the hippocampus has received extensive critical investigation (Rogers, Renoir, & Hannan, 2017). As well as its role in learning and memory, the hippocampus is involved with cognitive and emotional processing and is highly connected to other subcortical regions such as the amygdala which forms part of the limbic system. The hippocampus is densely innervated by serotonin (5-HT) projections from the dorsal raphe and serotonergic signalling is thought to play a key role in cognitive and emotional processing as well as being strongly implicated in a number of psychiatric disorders including depression (Coppen, 1967). It is also connected to the hypothalamus which forms part of the stress-related hypothalamic-adrenal-pituitary axis (HPA axis) which is known to be perturbed in depression including; causing elevated levels of stress and circulating cortisol (Burke, Davis, Otte, & Mohr, 2005; Parker, Schatzberg, & Lyons, 2003). Moreover, functionally relevant levels of hippocampal neurogenesis have been demonstrated in the adult human (Spalding et al., 2013) which has been implicated in antidepressant response (Phillips, Batten, Tremblay, Aldosary, & Blier, 2015), stress response and cognitive function (O'Leary & Cryan, 2014). Therefore the hippocampus is a prime structure for investigation in relation to major depression.

A large number of MRI studies have measured hippocampal volume in depression. For example, several meta-analyses of sMRI studies have reported an overall reduction in hippocampal volume in both hemispheres of patients with unipolar depression compared to

healthy controls (Arnone, McIntosh, Ebmeier, Munafo, & Anderson, 2012; Cole, Costafreda, McGuffin, & Fu, 2011; Gerritsen et al., 2011; Kempton, Salvador, Munafo, et al., 2011; Koolschijn et al., 2009; MacQueen et al., 2003). Specifically, a meta-analysis conducted by Videbech and Ravnkilde (2004) reported an 8% reduction in left hippocampus and 10% reduction in right hippocampus (Videbech & Ravnkilde, 2004). According to the neurotoxicity hypothesis, a reduction in hippocampal volume is a cumulative result of experiencing depression, PTSD and/or chronic stress (Sheline, 2011) and is an outcome of the onset of clinical symptoms. Evidence for this comes from Sheline and colleagues (1999) who found that reduced hippocampal volume was associated with increased duration of depression (Sheline, Sanghavi, Mintun, & Gado, 1999) and a meta-analysis of MRI studies of depression reported reductions in hippocampal volume may only be found in patients with multiple episodes or a long duration of illness (McKinnon, Yucel, Nazarov, & MacQueen, 2009). The proposed mechanism for this is that there is increased secretion of glucocorticoids (e.g. cortisol) by the HPA axis, often triggered by a stressor, which has excitotoxic effects on hippocampal neurones via apoptosis or inhibition of neurogenesis (Sapolsky, 2000). Reductions in neurotrophic factors (particularly Brain-Derived Neurotrophic Factor (BDNF)) are also thought to contribute to hippocampal volume loss (Duman & Monteggia, 2006). The hippocampus is therefore postulated to be involved in the pathophysiology of depression.

However, other sMRI studies of depression have reported right hippocampus only deficits (Bell-McGinty et al., 2002), left hippocampus only deficits (Mervaala et al., 2000) or no deficits at all (Posener et al., 2003). Inconsistencies in findings between studies of hippocampal volume changes in depression may be due to varying methodology (scanning protocols and delineation of hippocampal boundaries) or due to participant characteristics (Videbech & Ravnkilde, 2004). For example, hippocampal volume has been reported to decrease in healthy males with increasing age, but not in healthy females (Pruessner, Collins,

Pruessner, & Evans, 2001) and has been shown to differ between male and female participants with first-episode depression (Frodl et al., 2002).

Alternatively, the discrepancies between studies could be due to regional, rather than global hippocampal reductions. For example, recent advances in MRI analyses have allowed researchers to investigate changes in hippocampus morphology and a review of studies reporting differences in sub-regions of the hippocampus reported an overall localisation of abnormalities in the posterior hippocampus which may be more prominent than global hippocampal differences in depression (Malykhin & Coupland, 2015). By segmenting the hippocampus into anterior and posterior regions and using a volumetric approach, Neumeister and colleagues (2005) reported smaller posterior hippocampus (body and tail) bilaterally in remitted patients with recurrent depression (Neumeister et al., 2005). Reduced hippocampal volume was also found to be limited primarily to the tail of the hippocampus in patients with treatment resistant depression, particularly in females (Maller, Daskalakis, & Fitzgerald, 2007). Using vertex analysis, a recent study by Watanabe and colleagues (2017) identified significant inward deformations over the CA1 field and subiculum or ‘tail’ in bilateral hippocampus in first episode treatment-naïve patients compared to controls, which correlated with high salivary cortisol levels (Watanabe et al., 2017). Another study identified no significant global hippocampal volume differences in patients with depression but did report an abnormal shape corresponding to an inward deformation over the subiculum compared to healthy controls (Posener et al., 2003). The posterior region is known in particular to have projections with emotion processing regions including the amygdala and prefrontal cortex, and so alterations in this hippocampal region may be particularly important to the aetiology of depression.

Taken together, volume reductions have been observed in a range of brain regions associated with emotion, cognition as well as the stress response. In particular, reductions in global hippocampal volume have been widely observed in depression although there has been

a rise in studies that report morphological changes in sub-regions of the hippocampus, particularly the posterior hippocampus (tail). These abnormalities are thought to be associated with the pathophysiology of depression although the causal nature of this association is unclear.

## 4.9. Resting-state networks

As well as examining brain regions that are functionally activated during a task using fMRI, networks of brain regions that are active at rest have also been studied. Neuronal activity patterns in anatomically distinct brain regions are co-activated at rest and are therefore functionally connected (Friston, Frith, Liddle, & Frackowiak, 1993). These resting-state networks are thought to reflect functional communication between brain regions and can provide information that relates to human behaviour. Various functionally-connected networks have been defined. The best studied network is the Default Mode Network (DMN) although other networks include sensorimotor, visual, auditory, fronto-parietal, temporo-parietal and executive control networks.

### 4.9.1. Resting-state differences in depression using rsfMRI

A number of differences in resting-state networks have been reported in major depression including in the DMN, central executive network and salience network (Brakowski et al., 2017). In particular, converging evidence suggests that depression is associated with disruption to the DMN. This network is preferentially activated when the participant is internally focused; for example, during daydreaming, future planning, memory retrieval and thinking from different perspectives (Raichle et al., 2001). A number of brain regions have been consistently associated with the DMN. These include the posterior cingulate cortex

(PCC)/precuneus, medial prefrontal cortex (mPFC), ventral anterior cingulate cortex (ACC), and inferolateral parietal cortex (Dutta, McKie, & Deakin, 2015). The literature describes a dissociation between the anterior (centred on the mPFC) and posterior regions (centred on the PCC and inferolateral parietal lobes) of the DMN (Zhou et al., 2010). The anterior DMN is implicated in self-referential processing and emotion regulation whereas the posterior DMN is implicated in consciousness and memory processing (Zhu et al., 2012).

In particular, significant alterations in the DMN have been reported in major depression although these alterations in functional connectivity have been inconsistent. In general, studies that have investigated the anterior DMN have reported increased connectivity within this network. For example, increased connectivity within the subgenual ACC, orbitofrontal cortex, precuneus and thalamus has been reported in medicated depressed patients using the ICA approach (Greicius et al., 2007). Increased functional connectivity was also observed in PCC and mPFC in depressed patients (Sheline, Price, Yan, & Mintun, 2010; Zhou et al., 2010). Similar findings of increased connectivity within the anterior DMN (mPFC and vACC) were reported in medication-naïve depressed individuals as well, and this was positively correlated with participant's rumination score (Zhu et al., 2012). The anterior DMN has strong connections with limbic regions such as the amygdala and is implicated in self-referential processing and emotion regulation. Increased connectivity within the anterior DMN may therefore represent impaired emotion processing leading to rumination; characteristics commonly associated with major depression. In relation to the posterior DMN, some studies have reported increased functional connectivity in depressed patients; for example, Li and colleagues reported increased functional connectivity in the posterior sub-network in patients with depression which then normalised after antidepressant treatment (Li et al., 2013). On the other hand, reduced functional connectivity has been reported between the PPC/precuneus and bilateral caudate in subjects with first episode, unmedicated MDD compared to healthy controls (Bluhm et al.,

2009). It has been suggested that this altered connectivity is involved in the symptom of anhedonia in depression because of the role of the striatum (including the caudate) in processing pleasure and reward. Similarly, decreased functional connectivity in posterior DMN was replicated in a separate sample of treatment-naïve patients with depression and this was associated with the participant's over-general memory (Zhu et al., 2012). This is likely because the posterior DMN has strong connections with the hippocampus and is implicated in memory processing. Discrepancies between studies could be related to differences in sample sizes, medication status and analysis methods (ICA vs. seed-based), but also could be due to the heterogeneous nature of depression.

Taken together, abnormalities in a number of resting-state networks have been reported in depression. In particular, differences in connectivity within anterior and posterior regions of the DMN have been identified in patients with depression compared with healthy controls. However, the existing depression literature paints a confusing picture of increased or decreased connectivity within this network and whether altered DMN connectivity exists before the onset of depression is unclear.

#### 4.10. The neurotransmitter system and neurometabolites

As well as examining functions of brain regions using fMRI BOLD, proton MRS has been used to measure a range of endogenous chemicals. These chemicals are known to enable communication between brain regions via neurotransmission and neurometabolism and can provide information that relates to human behaviour and psychiatric disease. Many studies have focused on the two major excitatory and inhibitory neurotransmitters; glutamate and GABA as they have important roles in regulating cortical excitability. These neurotransmitters transmit signals across synapses between neurones, and are then recycled by astrocytes back into the

presynaptic neurone for reuse. Neurotransmitters that are not as abundant in the human brain such as dopamine or serotonin cannot be measured using this technique due to poor sensitivity.

#### 4.10.1. In-vivo metabolite differences in depression using proton MRS

Proton MRS studies of depression have shown abnormalities in a number of different neurometabolites such as GABA, NAA, glutamate/glutamine, GSH and choline in different regions of the brain. Many studies have focused on glutamate and GABA because they have important roles in regulating cortical excitability, and changes in glutamate and GABA metabolism and an imbalance of excitatory and inhibitory neurotransmission have been implicated in the pathophysiology of depression (Sanacora, 2010; Sanacora, Zarate, Krystal, & Manji, 2008; Taylor, Bhagwagar, Cowen, & Sharp, 2003). For example, diminished levels of GABA in peripheral blood plasma (Lu et al., 2014) and cerebrospinal fluid (CSF) (Kasa et al., 1982; Mann et al., 2014) have been reported in depressed patients compared to controls. In human post-mortem studies, down-regulation of genes coding for GABA interneuron-related peptides in specific brain regions (including the amygdala) of depressed patients have been identified (Guilloux et al., 2012) suggesting primary deficits in the GABA system. Moreover, interactions between GABA and 5HT (serotonin) pathways have been described suggesting an overlap with the serotonin theory of depression (a prominent theory that describes low levels of serotonin as a cause for depression which has led to the leading type of antidepressant treatment; selective serotonin reuptake inhibitors (SSRIs)) (Taylor et al., 2003). Complimentary to this, proton MRS has been used to assess neurometabolites in vivo. However, studies investigating brain biochemistry in depression using this technique have yielded largely inconsistent findings.



Perhaps one of the more consistent findings is a reduction in GABA in depressed patients, particularly in the occipital cortex where GABA is easier to measure using current MRS methodology. For example, a recent meta-analysis by Romeo and colleagues (2018) reported an overall reduction in GABA levels in unmedicated depressed patients (major depression and bipolar depression) compared to controls (Romeo, Choucha, Fossati, & Rotge, 2018). In some studies this has been quantified as a 20% - 50% reduction in GABA in the occipital cortex (Mason et al.; Sanacora et al., 2004; Sanacora et al., 1999) and decreases have also been reported in prefrontal regions (Hasler et al., 2007). Treatments for depression such as SSRIs and electroconvulsive therapy (ECT) have also been shown to increase occipital GABA (Sanacora et al., 2003; Sanacora, Mason, Rothman, & Krystal, 2002) along with improving depressive symptoms. However, the presence of these abnormalities into remission is still unclear as some studies suggest occipital GABA levels remain low into remission (Bhagwagar et al., 2007) whereas others suggest no differences are present in comparison with healthy controls (Shaw et al., 2013). It could be that discrepancies between findings represent differences in age, nature of depression (recurrent vs. single-episode depression) and symptom severity (Sanacora et al., 2004).

Similarly, MRS studies of depression largely report reduced glutamate concentrations, particularly in anterior regions of the brain (Niciu, Ionescu, Richards, & Zarate, 2014; Yuksel & Ongur, 2010). For example, a decrease in glutamate/glutamine or Glx (a combination of glutamate and glutamine detected at lower field strengths) levels have been reported in the prefrontal cortex (Hasler et al., 2007; Michael et al., 2003a), anterior cingulate cortex (Auer et al., 2000; Zhang et al., 2013), left amygdala (Michael et al., 2003b) and hippocampus (Block et al., 2009) of depressed patients compared to controls which has been shown to normalise in response to ECT and/or antidepressant treatment (Michael et al., 2003a, 2003b; Zhang et al., 2013). However, other studies have reported no differences in Glu/Gln/Glx levels, including in

the ACC of acutely depressed patients (Price et al., 2009), in the hippocampus of first-episode and recurrently depressed individuals (Milne, MacQueen, Yucel, Soreni, & Hall, 2009) and in the DMPFC and VMPFC of remitted patients (Hasler et al., 2005). Finally, one study reported an elevation in Glx levels in the DLPFC in a sample of older (over 60 years) female participants (Binesh, Kumar, Hwang, Mintz, & Thomas, 2004). In the occipital cortex, evidence is limited and studies report a mixed picture of glutamate. Some studies of depressed patients and recovered unipolar and bipolar participants report increased concentrations of glutamate (or Glx) (Bhagwagar et al., 2007; Sanacora et al., 2004) whereas other studies report no differences at all (Godlewska, Near, & Cowen, 2015; Murrough et al., 2010; Price et al., 2009). However, one factor that is important to consider when comparing MRS studies of glutamate is that some studies report Glx (composite measure) which could indicate increases in glutamate, glutamine or both. Therefore, decomposing glutamate levels in depression is difficult due to differences in medication status, symptom severity, measurements in different regions of the brain and reports of different measures of glutamate.

Another metabolite that has been investigated in relation to depression is NAA. This molecule produces the largest signal in the MR spectrum and is therefore easily quantifiable using MRS even at lower field strength (e.g. 1.5 T). NAA is thought to represent neuronal viability and reductions can be an index of neuronal loss or dysfunction (Demougeot, Marie, Giroud, & Beley, 2004). Again, studies have provided discrepant findings. For example, in the basal ganglia, most studies have reported no significant changes in NAA of depressed patients compared to controls (Hamakawa, Kato, Murashita, & Kato, 1998), although a few studies have reported decreased levels in the thalamus (Mu et al., 2007) and caudate (Vythilingam et al., 2003). Similarly, studies have reported no differences in NAA in the ACC (Auer et al., 2000; Kumar et al., 2002; Pfliderer et al., 2003), hippocampus (Ende, Braus, Walter, Weber-Fahr, & Henn, 2000) and prefrontal cortex (Brambilla et al., 2005; Coupland et al., 2005; Farchione,

Moore, & Rosenberg, 2002; Michael et al., 2003a) although other studies have reported reduced levels in the prefrontal region of depressed patients (Gruber et al., 2003) and moderately depressed individuals (Sözeri-Varma, Kalkan-Oğuzhanoglu, Efe, Kiroglu, & Duman, 2013) compared to healthy matched controls. The evidence for altered NAA levels in the occipital cortex is limited although one study reported reduced levels in participants that had recovered from depression (Bhagwagar et al., 2007) and another study reported no differences (Sanacora et al., 2004). However, one factor that is important to consider when measuring NAA levels in the brain is that there is evidence that this molecule is altered by antidepressant medication (Gonul et al., 2006) and could therefore account for some of the variability in findings between studies.

Taken together, a large corpus of research has investigated differences in neurometabolites in numerous regions of the brain in depression. In particular, alterations in glutamate and GABA have been reported in depression using proton MRS including reductions in GABA and increases in glutamate in the occipital cortex. Changes in NAA could also be related to the depressed state although all three neurometabolites seem to be influenced by medication status, symptom severity and patient heterogeneity. Methodological factors associated with MRS (e.g. obtaining good quality spectra, separating out glutamate and studying challenging brain regions) could also explain discrepancies between studies. For example, investigating glutamate and GABA changes in the amygdala (a key region that has shown to be dysfunctional in depression) is difficult because it is a small, subcortical structure that is in close proximity to air-tissue interfaces making it susceptible to poor SNR.

#### 4.11. Thesis investigation

The present studies set out to determine the neural mechanisms underlying vulnerability for depression using fMRI, structural MRI, resting-state MRI and MRS, to complement the

cognitive and behavioural mechanisms explored in previous chapters. A different sample of healthy individuals were recruited with a range of chronotypes, identified using the reduced Morningness Eveningness Questionnaire (Horne & Östberg, 1976). In order to separate out the neural mechanisms that underlie risk for depression in relation to chronotype, participants were excluded from participation if they self-reported a diagnosis of depression or history of depression, a biological parent with a diagnosis of depression or a diagnosed sleep disorder (both considered risk factors for depression). Participants were largely undergraduate or postgraduate students at the time of recruitment.

Four cross-sectional experiments were designed to probe structural and functional differences in relation to chronotype, that have been previously been reported in depressed patients. First, fMRI was used to examine amygdala reactivity to negative facial expressions. Second, MRI was used to investigate structural differences in the hippocampus. Third, resting-state fMRI was used to examine differences in functional connectivity within the default mode network and fourth, proton MRS was used to detect potential changes in neurometabolites (e.g. GABA) in the occipital cortex.

This project was approved by the University of Roehampton Ethics Committee (ref: PSYC 15/198) and was carried out in accordance with the latest version of the Declaration of Helsinki (World Med, 2013). Details of the methodology and results of each study are reported in subsequent chapters. The final chapter will provide a general discussion of the findings across all six experiments plus limitations and implication for future research.

# **CHAPTER FIVE**

## **Neural substrates involved with processing negative facial expressions**

### **5.1. Introduction**

As described in Chapter 4, functional MRI studies of major depression have reported widespread abnormalities in emotion processing and regulation circuitry. In particular, the amygdala has been a structure of interest because it is thought to modulate attention towards an emotional stimulus and generate an emotional response, and studies of depression have consistently reported an elevated and sustained response to negative emotional information including facial expressions (Disner et al., 2011). In addition, a number of functional impairments have been observed in prefrontal brain regions implicated in top-down inhibition of the amygdala in depression including the dACC (Dannlowski et al., 2009; Davidson et al., 2002). In order to investigate whether these aberrant neural responses are present before the onset of depression, it is necessary to examine neuroimaging data in never-depressed individuals at increased risk for developing depression.

A growing corpus has identified populations at-risk for developing depression with functional abnormalities in emotion processing brain regions. For example, high neuroticism (a recognised risk factor for depression) is associated with elevated right amygdala response to fearful vs. happy faces (Chan, Norbury, Goodwin, & Harmer, 2009). Herringa and colleagues (2016) reported a positive correlation between right amygdala response to negative vs. neutral

stimuli and childhood adversity (a recognised risk factor for depression) (Herringa et al., 2016). Monk *et al.*, reported increased activation in left and right amygdala to passive viewing of fearful faces (an affect not present when attention was constrained to a non-emotional component of the face (i.e. nose width) or to the subjective emotion experienced while viewing the stimuli) in the offspring of depressed parents (Monk et al., 2008). More recently, Mannie and colleagues (2011) found no difference between offspring of depressed parents and matched controls in amygdala reactivity to negative facial expressions during an emotion matching task (Mannie, Taylor, Harmer, Cowen, & Norbury, 2011) but did report reduced activation of frontal regions which may reflect perturbed regulation of aversive stimuli. A direct comparison across these at-risk studies is challenging due to differences in task parameters (implicit gender discrimination, passive viewing or emotion matching) and participant characteristics. Nevertheless, current data suggest abnormal processing of emotional information in never-depressed at-risk individuals.

In relation to emotional regulation, neuroimaging studies of never-depressed at-risk populations reveal similar functional connectivity impairments to depressed patients. For example, high neuroticism is associated with decreased functional connectivity between dACC and amygdala for sad compared to neutral faces (Cremers et al., 2010). Reduced functional connectivity between amygdala and rostral ACC has also been observed in carriers of the 5-HTTLPR polymorphism (Pezawas et al., 2005). Taken together, these data suggest that never-depressed at-risk individuals display abnormal amygdala reactivity to negative stimuli and altered cognitive control processes responsible for emotional regulation, which may reflect a neural vulnerability marker present prior to the onset of depression.

In sum, previous evidence suggests that patients with depression have an enhanced amygdala response to negative facial expressions (Disner et al., 2011) as compared to healthy individuals, which is attenuated with successful pharmacotherapy (Anand et al., 2007; Fu et al.,

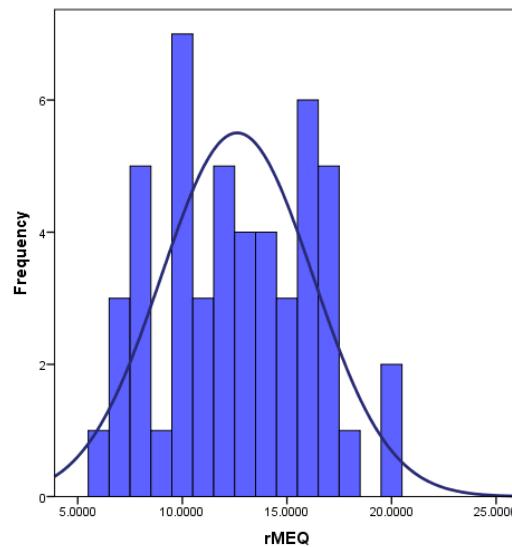
2004; Sheline et al., 2001). Other groups known to be at increased risk for developing depression also show a similar enhanced amygdala response to negative stimuli including; high neuroticism (Chan, Norbury, Goodwin, & Harmer, 2009), childhood adversity (Herringa et al., 2015) and offspring of depressed parents (Monk et al., 2008). To my best knowledge, however, no one has investigated amygdala reactivity to negative stimuli and related this to chronotype. Here it was hypothesised that late chronotype would be associated with increased amygdala reactivity to negative (fearful) facial expressions, similar to the pattern of activity seen in depressed patients and other at-risk groups. A second objective was to explore amygdala-fronto connectivity as both depression, and risk for depression, have been associated with impaired emotional regulation of the amygdala by higher cortical regions (Mayberg, 2003). Here, it was predicted that late chronotype would be associated with reduced connectivity between amygdala and brain regions implicated in emotion regulation.

## 5.2. Methods

### 5.2.1. Participants

The study was approved by the local ethics committee and written informed consent was obtained prior to any study procedures taking place. Participants were in good physical health and free of concurrent medication. Exclusion criteria were current or previous depression, presence of major depression in a biological parent, diagnosed sleep disorder (each assessed by self-report) and contraindication for MR examination. A total of 50 participants were recruited (38 females, age range 18-37 ( $M=21.24$ ,  $SD=3.77$ ) with a range of chronotypes (as shown in *Figure 11*). Chronotype was determined using the 5-item reduced Morningness-Eveningness Questionnaire (Adan & Almirall, 1991a), based on the full version of the MEQ validated for a young adult population (18-32 years). Sleep quality was assayed using the

Pittsburgh Sleep Quality Index (Buysse et al., 1989). Depression and anxiety were measured using the Patient Health Questionnaire-4 (Löwe et al., 2010). The time at which the MRI scan took place was agreed between the participant and investigator and recorded, but generally took place between 10am and 5pm.



*Figure 11. A frequency distribution diagram showing the distribution of chronotype scores in the sample. The data appear approximately normally distributed (tests of normality were non-significant) encompassing a range of rMEQ scores from 6 -20.*

### 5.2.2. Image data acquisition

All imaging data were acquired on a research dedicated 3T Magnetom TIM Trio (Siemens, Erlangen, Germany) fitted with a 32-channel head coil and located at the Combined Universities Brain Imaging Centre (CUBIC). For each participant a T<sub>1</sub>-weighted whole-brain scan (magnetization-prepared rapid acquisition with gradient echo (MPRAGE) was collected, inversion time (T<sub>I</sub>) = 1100 ms, repetition time (T<sub>R</sub>) = 1830 ms, echo time (T<sub>E</sub>) = 3.03 ms, flip



angle (FA) =  $11^\circ$ , field of view (FOV) =  $256 \times 256 \times 160 \text{ mm}^3$ , voxel size =  $1 \times 1 \times 1 \text{ mm}^3$ ). Functional MR data were acquired using a  $T_2^*$ -weighted echo planar imaging sequence (EPI,  $T_R = 2000 \text{ ms}$ ,  $T_E = 31 \text{ ms}$ , FA =  $85^\circ$ , FOV =  $192 \times 192 \times 87 \text{ mm}^3$  [29 slices, voxel size =  $3 \times 3 \times 3 \text{ mm}^3$ ], number of measurements = 170, imaging bandwidth = 752 Hz/px, GRAPPA acceleration factor = 2). Gradient echo field mapping data were also acquired for EPI off-resonance distortion correction ( $T_R = 400 \text{ ms}$ ,  $T_{E1} = 5.19 \text{ ms}$ ,  $T_{E2} = 7.65 \text{ ms}$ , flip angle =  $60^\circ$ , FOV =  $192 \times 192 \times 126 \text{ mm}^3$ , voxel size =  $3 \times 3 \times 3 \text{ mm}^3$ ).

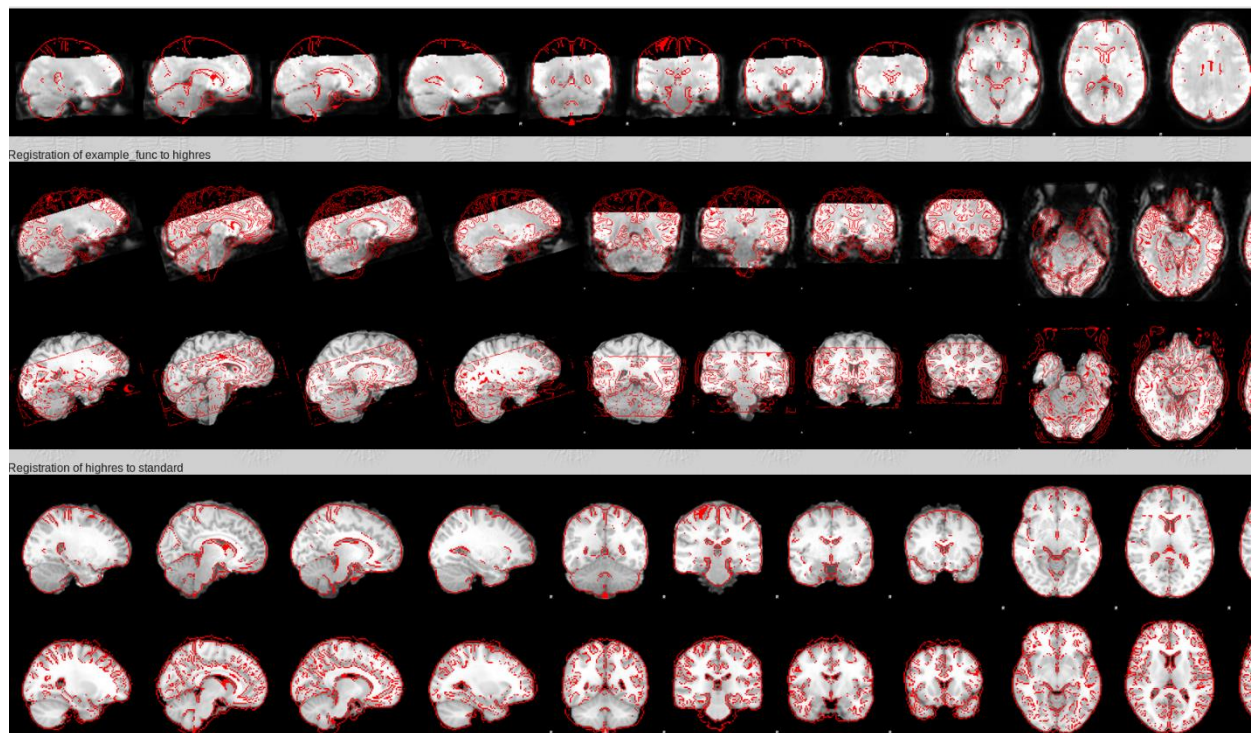
### 5.2.3. fMRI experimental task

During fMRI scanning, participants completed a well validated gender discrimination task involving the rapid presentation of greyscale fearful and happy faces taken from the NimStim database (Tottenham et al., 2009). Nine 20 second blocks of baseline (fixation cross) were interleaved with 8 blocks of the emotional faces (again 20 seconds in duration, 4 blocks of fearful and four blocks of happy faces). Individual faces were presented for 100ms and the participant had to indicate, by button press, the gender of the face. Equal numbers of male and female faces were presented in each condition. Stimuli were presented on a personal computer using E-Prime (using version 2.10.242, Psychology Software Tools Inc., USA) and projected onto an opaque screen at the foot of the scanner bore, which subjects viewed using an angled mirror mounted above the head coil. Both accuracy and response time were recorded by E-Prime.

### 5.2.4. fMRI analysis pipeline

All image pre-processing and analyses were performed using FSL version 5.0.10 (FMRIB Software Library, <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). The following pre-statistical

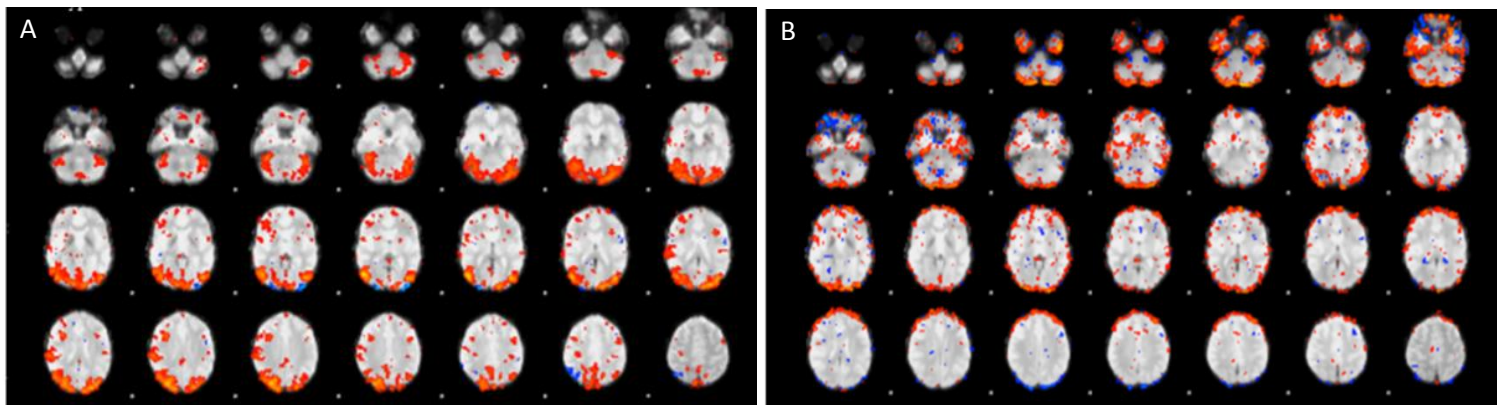
processes were applied to all fMRI data: non-brain removal; rigid-body motion alignment; high-pass temporal filtering (Gaussian-weighted least-squares fitting with frequency cut-off point = 80 s); correction of off-resonance geometric distortions in the EPI data using B0 field maps derived from the dual-echo gradient echo dataset; artifact removal based on probabilistic ICA (Independent Component Analysis – see below); spatial normalization to Montreal Neurological Institute (MNI152) 2 mm isotropic atlas space using boundary-based registration and non-linear registration (as shown in *Figure 12*) (during registration, signal loss, resulting from through-slice field gradients, was calculated and used as a cost function mask to exclude voxels where signal loss was greatest); Gaussian filtering (full width at half maximum (FWHM) = 5 mm).



*Figure 12. A summary report for one participant where fMRI images are registered to standard space in a two-stage process (top row). The low resolution image (fMRI image) is firstly registered to a high resolution image (structural image) using boundary based registration (second row). Then, the high resolution image is registered to standard (MNI) space using non-linear registration (third row).*

### 5.2.5. Controlling for structured noise

To control for structured noise, manual ICA-based artifact removal was conducted. The independent component maps for each participant were visually inspected to identify noise components based on both the spatial layout of the component maps and the power spectra of the associated time series (please see *Figure 13* for examples) (Griffanti et al., 2016). Variance uniquely associated to the components labelled as noise was subsequently regressed from each individual's data prior to statistical modelling.



*Figure 13. Example components from ICA analysis identified as signal (A) and noise (B).*

### 5.2.6. Analysis of functional imaging data

Analyses of the filtered data from individual subjects (first level analysis) were computed using the general linear model with local autocorrelation correction. Two regressors were defined (fearful and happy faces) and were convolved with a haemodynamic response function, using a variant of a  $\gamma$  function (i.e. a normalization of the probability density function of the  $\gamma$  function) with a standard deviation of 3 s and a mean lag of 6 s. In addition, temporal derivatives and estimated motion parameters (three translation and three rotation) were included in the model as regressors of no interest to increase statistical sensitivity.

At the group level, linear effects of chronotype on BOLD response in bilateral amygdala were tested for significance using non-parametric permutation tests (applying 5000 permutations). Control of the family-wise error rate was obtained using threshold-free cluster enhancement (Smith & Nichols, 2009). Left and right amygdala *a priori* regions of interest were taken from the Harvard-Oxford subcortical atlas distributed within FSL. This atlas is derived from T<sub>1</sub>-weighted images of 37 subjects (21 male, age range 18-50) and combined to form population probability maps for 21 subcortical structures including the amygdalae.

### 5.2.7. Psychophysiological interaction analysis

In a complementary analysis, a generalised psychophysiological interaction analysis (PPI) (Friston et al., 1997; McLaren, Ries, Xu, & Johnson, 2012) was conducted across the whole brain in order to explore how functional connectivity between brain regions varied with task. As a significant correlation between chronotype and BOLD signal was observed for both right and left amygdala (please see results), both regions were used separately as seed regions. At the individual level the PPI GLM analyses included the original task conditions (fear and happy faces), the mean time course from each cluster identified in the analyses described above, and the two interaction terms (fear faces x seed, happy faces x seed). Temporal derivatives and six estimated motion parameters were also included in the model. This analysis identified regions that displayed stronger functional connectivity with the left/right amygdala for fearful facial expressions compared to happy facial expressions.

At the group level, the contrast images for the PPI effects were entered along with chronotype as a regressor in a whole-brain analysis. Brain regions that showed connectivity with the amygdala were identified, correlating positively or negatively with chronotype (rMEQ

score). This was tested for significance using non-parametric permutation tests (applying 5000 permutations) and threshold-free cluster enhancement.

## 5.3. Results

### 5.3.1. Participants

Participant characteristics are presented in *Table 5*. Measures of anxiety ( $r_s(50) = 0.13$ ,  $p = .37$ ), depression ( $r_s(50) = 0.13$ ,  $p = .37$ ) and time of scan ( $r_s(50) = 0.021$ ,  $p = .88$ ) were not significantly correlated with rMEQ. No systematic bias was observed between the participant's chronotype and the time of day the experiment was performed as both early and late chronotype were equally likely to attend morning or afternoon/evening sessions (please see *Figure 14*). Chronotype scores were similar between male and female participants (independent samples  $t$ -test ( $t(13.73) = .68$ ,  $p = .51$ ). However, there was a significant correlation between rMEQ and PSQI score ( $r(50) = 0.38$ ,  $p = .006$ ) and age ( $r(50) = -0.33$ ,  $p = .018$ ) such that late chronotype was associated with better sleep quality and older age.

Table 5. Descriptive statistics and basic demographics.

Variable	M (SD)	Range (this sample)	Range (original scale)
Age	21.24 (3.77)	18-37	-
Gender	Female 38 (76%); Male 12 (24%)	-	-
Sleep quality	7.30 (3.42)	1 - 15	0 – 21
Chronotype	12.62(3.62)	6 - 20	4 – 25
PHQ-4 (Anxiety)	0.91 (1.09)	0 - 5	0 – 6
PHQ-4 (Mood)	1.61 (1.58)	0 - 6	0 -6

Note. Values show mean (SD). Also included are the questionnaire range (minimum-maximum) for the study sample and original scale. Sleep quality (PSQI = Pittsburgh Sleep Quality Index), chronotype (rMEQ = reduced Morningness Eveningness Questionnaire), mood and anxiety (PHQ-4 = Patient Health Questionnaire).

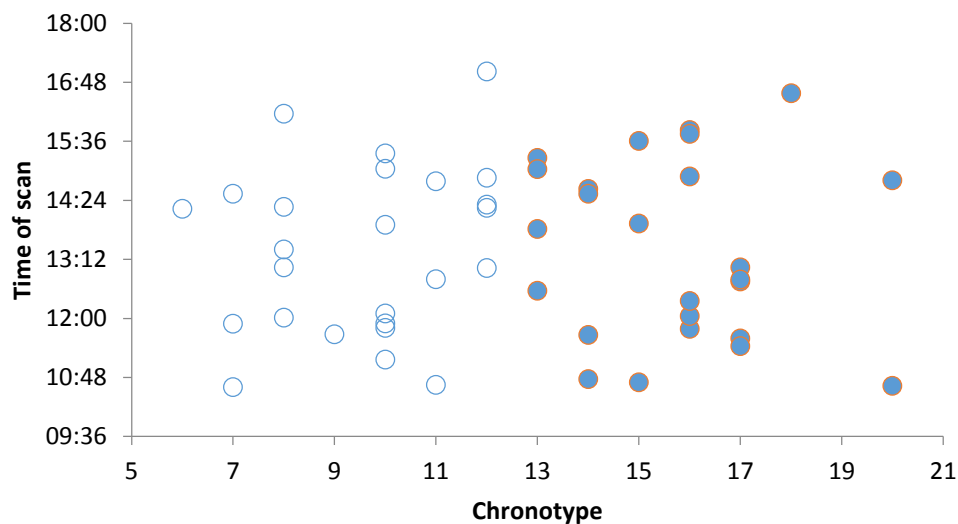


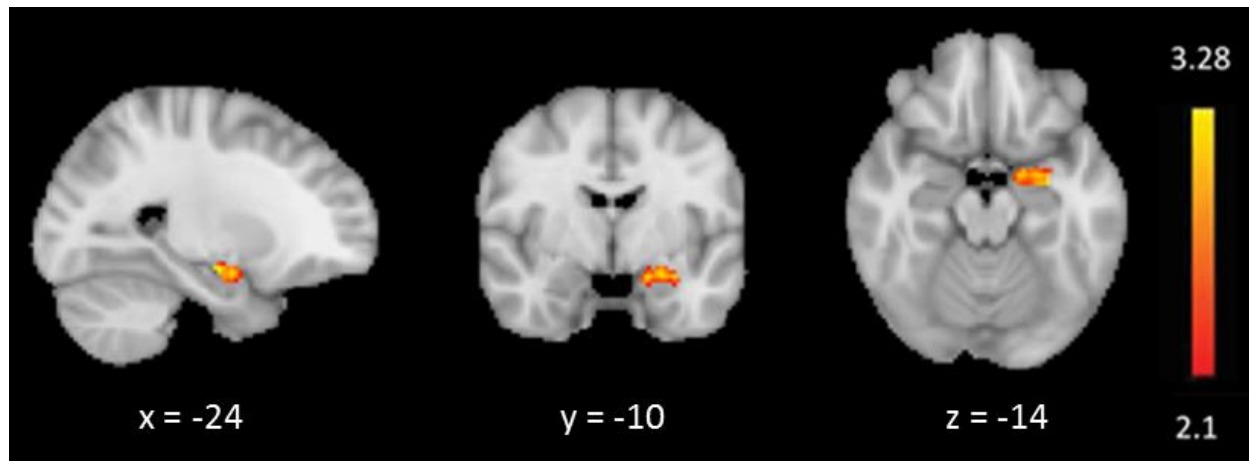
Figure 14. Scatter plot showing no systematic bias between chronotype (early chronotype = closed circles, late chronotype = open circles, based on median split of data) and time of scan.

### 5.3.2. Behavioural results

Inspection of the behavioural data acquired during scanning indicated that participants were engaged with the task and were highly accurate to classify faces as male or female (> 85%) with discrimination accuracy for happy faces greater than fearful faces (fearful faces:  $M = 85.20\%$ ,  $SD = 9.84$ , happy faces:  $M = 88.70\%$ ,  $SD = 9.44$ , dependent samples  $t$ -test:  $t(45) = -3.41$ ,  $p = .001$ ). Response latencies to happy faces were similar to fearful faces (happy faces:  $M = 628.52$  ms,  $SD = 120.96$ , fearful faces:  $M = 642.50$  ms,  $SD = 132.52$ , dependent samples  $t$ -test:  $t(45) = 1.84$ ,  $p = .072$ ).

### 5.3.3. FMRI results

Using a region of interest approach, a significant negative correlation between BOLD response and chronotype in bilateral amygdala was observed (left amygdala  $x = -30$ ,  $y = -6$ ,  $z = -18$ , maximum  $t$ -value = 3.97; cluster size = 198 voxels,  $p < .001$ , [please see *Figure 15*]; right amygdala  $x = 32$ ,  $y = 2$ ,  $z = -20$ , maximum  $t$ -value = 3.20; cluster size = 91 voxels,  $p = .012$ ) such that participants with higher rMEQ scores (increased early chronotype) showed reduced activity to fearful vs. happy faces in left and right amygdalae. There were no regions that exhibited a positive association between chronotype and BOLD response. In a further exploratory analysis, chronotype was regressed across the whole brain but no other regions were found to be significantly associated.



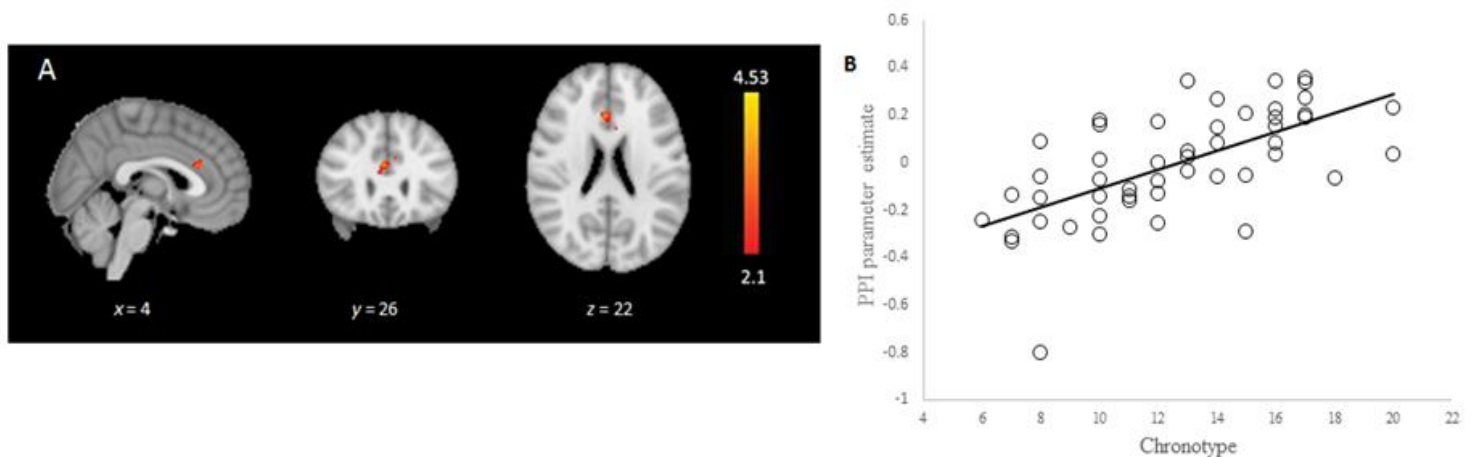
*Figure 15. BOLD correlates with rMEQ in left and right amygdala (left amygdala displayed). Early chronotype is associated with reduced BOLD response to fear vs. happy faces. Lower numerals refer to coordinates in Montreal Neurological Institute (MNI) space. Colour bar and numerals:  $t$  value range.*

In a further analysis, restricted to the functional clusters observed above, PSQI score, gender, age, mood and anxiety levels (computed as the sum score for the mood and anxiety components of the PHQ-4) and time of scan (i.e. the start time of the experiment as recorded by the stimulus presentation software) were included as additional covariates. Bilateral amygdala activation remained significant although the amplitude and extent of activation was reduced (left amygdala  $x = -24$ ,  $y = -10$ ,  $z = -14$ , maximum  $t$ -value = 3.17; cluster size = 55 voxels,  $p = .018$ ; right amygdala  $x = 28$ ,  $y = -4$ ,  $z = -14$ , maximum  $t$ -value = 2.92; cluster size = 61 voxels,  $p = .014$ ). This suggests the negative association observed between rMEQ and BOLD response is robust, and largely independent of sleep quality, gender, age, mood, anxiety levels and time of scan.



#### 5.3.4. PPI analysis results

The PPI analysis revealed a positive association between chronotype (rMEQ) scores and right amygdala – dACC coupling ( $x = 4, y = 26, z = 22$ , maximum t-value = 4.53; cluster size = 54 voxels,  $p = .026$ ) and right frontal pole ( $x = 34, y = 46, z = -2$ , maximum t-value = 5.16; cluster size = 40 voxels,  $p = .02$ ) when viewing fearful faces compared to happy faces (please see *Figure 16*). As above (FMRI results) PSQI score, gender, age, mood and anxiety levels and time of scan were included as additional covariates to assess the specificity of this effect. Both the dACC cluster ( $x = 4, y = 26, z = 22$ , maximum t-value = 3.77; cluster size = 33 voxels,  $p < .05$ ) and frontal pole clusters ( $x = 34, y = 48, z = -2$ , maximum t-value = 4.17; cluster size = 40 voxels,  $p < .05$ ) remained significant. This finding indicates that late chronotype (lower rMEQ scores) is associated with reduced functional coupling between right amygdala and dACC for fearful vs. happy facial expressions even after accounting for a number of possible confounds.



*Figure 16. PPI analysis displaying A) brain regions identified as showing functional connectivity with the right amygdala that are positively correlated with chronotype (rMEQ) scores in response to fearful vs. happy facial expressions (colour bar and numerals as in Figure 15), and B) scatter plot showing positive association between right amygdala – ACC connectivity and chronotype scores.*

## 5.4. Discussion

This is the first study, to my knowledge, to explore the neural basis of emotional processing biases towards negative facial expressions and relate this to chronotype. A significant negative correlation was observed between BOLD response in bilateral amygdalae and rMEQ score such that late chronotype (lower rMEQ score) was associated with increased BOLD response to fearful vs. happy faces. Moreover, a positive correlation between rMEQ score and functional connectivity between right amygdala and dACC was observed where early chronotype was associated with increased connectivity in response to fearful vs. happy faces. These effects were present independent of current or previous diagnosis of depression or sleep disorder, and were not driven by sleep quality, age, gender, measures of mood and anxiety or time of scan. Previous evidence has identified late chronotype to be associated with increased prevalence of depression (Antypa et al., 2016; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) and hyperactivity of the amygdala to negative stimuli has been reported in depressed patients (Sheline et al., 2001) and individuals at increased risk for depression (Chan et al., 2009). Impaired emotional regulation has also been observed in depressed individuals (Erk et al., 2010) and never-depressed at-risk populations (Cremers et al., 2010; Pezawas et al., 2005). Here it was observed that late chronotype was associated with hyperactive amygdalae and reduced amygdala-ACC connectivity to negative faces which may reflect the neural processes underlying vulnerability to depression in late chronotype individuals.

Biases in facial expression recognition have been reported in individuals with depression involving a discrimination bias towards negative emotions and/or away from positive emotions. For example, Gur and colleagues reported reduced sensitivity to recognise happy facial expressions and an increased likelihood to misclassify neutral faces as sad in a sample of depressed patients (Gur et al., 1992). Additionally, depressed patients have been

shown to have reduced spatial attention towards positive facial expressions during a face-in-the-crowd task (Suslow, Junghanns, & Arolt, 2001). In a longitudinal study, patients were more likely to report ambiguous faces as being negative when they were first admitted as depressed than in a remitted state, and patients were more likely to relapse after six months if they perceived faces as being more negative at admission or discharge (Bouhuys et al., 1999). This finding highlights the significance of negative biases in predicting relapse as theorists suggest that these negative biases play a key role in the aetiology and maintenance of depressed states. It is therefore important to develop prevention strategies that aim to identify and remove negative biases; for example, using psychological therapies (e.g. Cognitive Behavioural Therapy) to reverse these cognitions.

Similar biases have also been reported in remitted depressed patients (Anderson et al., 2011; Bhagwagar, Cowen, Goodwin, & Harmer, 2004) thereby suggesting that negative biases may either signal a ‘scar’ effect (arise as a consequence of previous depression) or a pre-existing cognitive vulnerability. However, negative biases have also been reported in never-depressed individuals at increased risk for developing depression; for example, Chan and colleagues (2007) reported an increased threshold to recognise happy facial expressions in highly neurotic individuals as compared to healthy controls (Chan, Goodwin, & Harmer, 2007). As discussed in Chapters 2 and 3 (and in Berdynaj et al., 2016), late chronotypes have also been shown to display increased recognition of sad facial expressions and decreased recognition of happy facial expressions. Taken together, these data suggest that negative biases are present in never-depressed at-risk individuals and may, therefore, reflect a vulnerability marker for depression in these groups.

The amygdala has been shown to play a key role in both facial expression recognition and depression. For example, increased activation in the left amygdala in response to masked emotional (both fearful and sad) facial expressions (Sheline et al., 2001) as well as facial

expressions morphed between intensities of sadness (Fu et al., 2004) have been reported in depressed patients which normalised with antidepressant treatment. Surguladze and colleagues also reported a linear increase in activation in the left amygdala of depressed individuals in response to increasing expressions of sadness (Surguladze et al., 2005). Using a meta-analytic approach to pool data across numerous functional neuroimaging studies (44 in total), Groenewold and colleagues concluded that depressed patients display hyper-activation to negative stimuli and reduced activation to positive stimuli in left and right amygdala; a pattern of activation consistent with the negative biases widely reported in depression (Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013). Aberrant amygdala responses to negative facial expressions have also been associated with never-depressed at-risk groups; including high neuroticism (Chan et al., 2009), childhood adversity (Herringa et al., 2015) and offspring of depressed biological parents (Monk et al., 2008). Here, similar findings are reported in never-depressed late chronotypes.

The nature of the current study i.e. an implicit facial recognition task, also suggests that the aberrant amygdala responses observed in the current study are related to maladaptive implicit processing of negative facial expressions. This is similar to other studies using implicit processing tasks e.g. an unconscious masked faces paradigm (Sheline et al., 2001) and implicit sad facial expressions (Fu et al., 2004) reporting enhanced amygdala reactivity in depressed patients. It has been suggested that hyperactivity of the limbic system (particularly the amygdala) generates a bottom-up signal which suppresses higher cortical areas responsible for processing emotional information resulting in maladaptive interpretations of the environment and social interactions (Disner et al., 2011). This may therefore explain the neural basis for the negative biases observed in depressed and at-risk populations. For example, Mannie and colleagues reported participants with a biologically depressed parent displayed no biases for personality descriptors but an overall increased reaction time, suggesting a fault in the initial

coding of emotionally valenced words (Mannie, Bristow, et al., 2007). Together, these findings suggest that a heightened amygdala response to negative affective stimuli may explain an increased risk for major depressive disorder, including late chronotype individuals.

The heightened amygdala response observed in late chronotypes was also associated with reduced functional connectivity with the dACC. This finding is in accordance with previous evidence that depressed patients show reduced dACC-amygdala functional connectivity in response to negative (angry and sad) vs. neutral facial expressions (Dannlowski et al., 2009). Moreover, reduced ACC-amygdala connectivity has also been observed in never-depressed at-risk populations including high neuroticism (Cremers et al., 2010) and individuals with a genetic risk of depression (Pezawas et al., 2005). As reviewed by Disner et al (2011), cognitive biases observed in depression appear to be influenced by: 1) neurobiological processes that initiate the cognitive bias, and 2) reduced cognitive control, which allows the bias to continue (Disner et al., 2011). The dACC is part of a network of higher cortical areas including the prefrontal cortex (PFC); medial and lateral orbitofrontal cortex involved in the cognitive regulation of limbic regions associated with processing emotion. In healthy controls, the dACC has been shown to be involved with down regulation of negative emotions and modulation of the neural activity of the amygdala (Mak et al., 2009; Phan et al., 2005). The dACC also plays a critical role in monitoring and adjusting emotional reactivity and cognitive control (Botvinick et al., 2001; Kerns et al., 2004; Pizzagalli, 2011), and has been shown to be hypoactive in major depression (Davidson et al., 2002). It has been suggested that higher cortical areas responsible for suppressing task-irrelevant information using a ‘top-down’ mechanism may be altered in depression. For example, Etkin and colleagues demonstrated top-down inhibition of amygdala activity by the rostral ACC during an emotional conflict task using dynamic causal modelling (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). Although the directionality of the effect cannot be determined in the current study, the reduced connectivity

observed between the dACC and amygdala may therefore support the notion of impaired top-down regulation of the amygdala response by the dACC in late chronotypes. Of note, Rosenberg and colleagues (Rosenberg, Maximov, Reske, Grinberg, & Shah, 2014) reported significantly lower fractional anisotropy (FA; a measure of microstructural integrity) in white matter underlying the left ACC in healthy males free of current or previous psychiatric disorder characterised as late chronotypes as compared to early and intermediate types. In depressed patients, cingulate FA predicts remission (Korgaonkar, Williams, Song, Usherwood, & Grieve, 2014) and ACC white matter abnormalities have been reported in elderly depressed patients which affected cognitive functions and emotion modulation (Alexopoulos, Kiesses, Choi, Murphy, & Lim, 2002; Ballmaier et al., 2004). By contrast, Olvet et al., (Olvet et al., 2016) found no difference in cingulate FA values between depressed patients and healthy controls. The lack of consensus in studies of depressed patients may reflect the heterogeneous nature of the disorder. Emerging evidence in late chronotypes (Rosenberg et al., 2014) indicates reduced microstructural integrity of the ACC which could relate to abnormal suppression of the amygdala response, although future studies are needed to directly investigate this.

Previous neuroimaging studies show altered emotional regulation in depressed patients. For example, Erk and colleagues reported reduced functional connectivity between DLPFC and amygdala when depressed participants down-regulated negative images compared to healthy controls (Erk et al., 2010). The ability to down-regulate the negative emotion was also negatively correlated with the participant's HAMD (Hamilton Rating Scale for Depression) score. Beauregard and colleagues reported enhanced activity in dACC, right anterior temporal pole, right amygdala and right insular when depressed participants were asked to down-regulate their emotions whilst viewing sad films (Beauregard, Paquette, & Levesque, 2006), and Johnstone and colleagues reported increased activity in right PFC and ventro-lateral prefrontal cortex (VLPFC) of depressed participants during reappraisal of negative images (Johnstone,

van Reekum, Urry, Kalin, & Davidson, 2007). Both studies report enhanced activation of higher cortical regions involved in emotion regulation circuitry showing less efficient engagement of these regions.

Behaviourally, there is also evidence to suggest that major depression is associated with impaired emotion regulation. For example, Joormann and colleagues (2010) reported that depressed patients display a lack of inhibition of negative material during a negative affective priming task, which was associated with greater rumination i.e. a maladaptive process of ‘recycling’ thoughts (Joormann & Gotlib, 2010). In the same study, reduced inhibition of negative materials was also related to less use of cognitive reappraisal; a beneficial emotional regulation strategy involving re-interpreting the meaning of an emotional situation, and more use of expressive suppression; a maladaptive strategy involving inhibiting the expression of an emotion (Joormann & Gotlib, 2010). The misuse of these emotional regulation strategies, in particular rumination, has been shown to be important in the recurrence of depressive episodes and to some extent the chronicity of depressive disorders (Nolen-Hoeksema, 2000). Similarly, at-risk populations display impaired emotional regulation processes. For example, decreased thought suppression (a strategy to inhibit unwanted and intrusive thoughts) and increased rumination have been reported to mediate the association between high neuroticism and depression (Lu, Yang, Zhang, & Qiu, 2017). In relation to chronotype, Antypa and colleagues found cognitive reactivity (the activation of negative thoughts in response to low mood) and rumination to be mediators of the association between late chronotype and depression, independent of insomnia and neuroticism (Antypa et al., 2017). Moreover, late chronotype was recently found to be associated with increased expressive suppression whilst early chronotype was associated with increased cognitive reappraisal after controlling for age, gender, depressive symptoms, neuroticism and sleep quality (Van den Berg et al., 2018; Watts & Norbury, 2017). Plus, evening and intermediate types report reduced self-control of thoughts, emotions,

impulses, performance regulation and habit breaking (as measured by the Self-Control Scale) as compared to early chronotypes (Wang & Hu, 2016). Although the current data does not address the hypothesis directly, the data (increased amygdala reactivity and decreased dACC-amygdala functional connectivity) and earlier findings of reduced emotion regulation (Antypa et al., 2017; Wang & Hu, 2016; Watts & Norbury, 2017) appear consistent with this model of bottom-up suppression of higher cortical areas and top-down regulation of limbic regions and could, in part, explain the increased vulnerability for depression in late chronotype individuals. However, future studies designed to directly investigate this model of emotional regulation are needed.

#### 5.4.1. Specific limitations

Interpretation of the current findings should take into consideration a number of limitations specific to this methodology. Firstly, scan times were not fixed relative to individual wake up times to ensure that participants were in similar circadian phase. This is of import as previous neuroimaging studies have reported chronotype specific and time-of-day dependent effects on BOLD response to a number of cognitive tasks. For example, Schmidt et al, (2015) using a parametric verbal working memory task observed greater thalamic BOLD in late chronotypes tested at their optimal time (10.5 hours after waking) whereas early chronotype had higher BOLD responses than late types in middle frontal gyrus when tested in the morning session. In both cases, these differences were present only during the most cognitively demanding component of the task (3-back), no chronotype by time effects were present the less challenging 0- or 2-back conditions (Schmidt et al., 2015). More recently and employing a response inhibition task, Song et al., (2017) observed greater activation across a network of regions implicated in inhibition execution in early vs late chronotypes tested during a morning session (08:00 – 12:00) as compared to a late session (19:00 – 21:00) (Song et al., 2017). In an



alternative approach designed to limit potential synchrony effects Reske et al., (2015) scanned participants performing a variable load attention-to-motion task at a fixed interval (between 10 and 12 hours) post individual waking time. During high-attentional load early and late chronotype, as compared to intermediate types, showed reduced BOLD in right dorsolateral prefrontal cortex. At moderate attentional load a more complex pattern emerged, early chronotypes had greater BOLD response in bilateral insula whereas late chronotypes showed reduced activation in right superior parietal cortex (Reske, Rosenberg, Plapp, Kellermann, & Shah, 2015). Using the same approach Rosenberg and colleagues (2015) explored chronotype effects on a semantic priming task. Across all contrasts reported late chronotypes, relative to early or intermediate types, showed increased activation in a number of anatomical locations previously implicated in semantic processing (Rosenberg, Reske, Warbrick, & Shah, 2015). The limited available evidence clearly point to both chronotype-specific and chronotype-by-time dependent effects on regional BOLD. However, whether these synchrony effects (Schmidt et al., 2015; Song et al., 2017) translate from cognitively demanding tasks to less demanding implicit emotional processing tasks as reported in current work is unknown. Here, including time of scan as a covariate did not impact on the pattern of results and confirmed that late chronotype is associated with increased amygdala response to negative stimuli and reduced fronto-limbic connectivity after controlling for a number of possible confounds. Future studies, however, would benefit from explicitly controlling scan time according to individual chronotype. High neuroticism trait was also not measured or excluded in participants which is another population proven to be at-risk of depression (Kendler, Gatz, et al., 2006). There is some evidence that low neuroticism and morningness are correlated (Duggan, Friedman, McDevitt, & Mednick, 2014), however it has previously been shown that biases in emotional processing are present in a similar sample of young adults despite no observable differences in neuroticism (Berdynaj et al., 2016). Also, there a number of hormones that show diurnal

variation (e.g. cortisol). As a blood assay was not conducted, the neuroendocrine effects on the current results cannot be excluded. Of particular note, repeated clinical observations have reported an association between acute depression and increased availability of cortisol (Cowen, 2010) and elevated levels of cortisol is associated with hyperactivity of the amygdala (Tafet & Nemeroff, 2016). Against this, Kudielka et al (2006) reported an increased cortisol awakening response in early vs. late chronotypes independent of sleep duration or awakening time (Kudielka, Federenko, Hellhammer, & Wust, 2006). Similarly, Maierova and colleagues (Maierova et al., 2016) observed higher overall concentrations of cortisol in early chronotypes tested across a period of many hours. In this context, the current observation of an association between late chronotype and increased activation in bilateral amygdala makes fluctuation in cortisol levels an unlikely explanation for these findings.

#### 5.4.2. Conclusion

In conclusion, a clear association was found between late chronotype and increased sensitivity to negative emotional facial expressions in bilateral amygdala. Late chronotype was also associated with reduced dACC-amygdala functional connectivity suggesting impaired emotional regulation circuitry. These findings suggest that late chronotype is associated with an altered neural signature similar to that seen in depressed individuals and other at-risk groups which could underlie vulnerability for the disorder.

# CHAPTER SIX

## Hippocampal structure

### 6.1 Introduction

As reported in Chapter 4, structural neuroimaging studies of major depression have reported widespread changes in cortical and subcortical structures associated with memory, emotion processing and the stress response. In particular, the hippocampus has been a major structure identified in the pathophysiology of depression because bilateral hippocampal volume reductions have been widely reported in patients with depression (Cole et al., 2011; Kempton, Salvador, Munafo, et al., 2011; Koolschijn et al., 2009; Videbech & Ravnkilde, 2004). For example, a large scale study of 8927 participants reported a 1.24% reduction in bilateral hippocampal volume in patients with depression compared to healthy controls (small effect size, Cohen's  $d = -0.14$ ) (Schmaal et al., 2015). The relationship between reduced hippocampal volume and depression may be due to stress as an increase in circulating glucocorticoids including cortisol, often triggered in depression, has been shown to be excitotoxic to hippocampal neurones (Sapolsky, 2000). However, the causal nature of this association is unclear (Sheline, 2011) as some studies report reduced hippocampal volume only in patients that have experienced multiple episodes of depression (McKinnon et al., 2009) whereas other studies report similar differences in first-episode patients (Frodl et al., 2002; MacMaster & Kusumakar, 2004). Differences in methods and participant characteristics (e.g. age, gender, medication status) may play a role in explaining the differences in findings between studies, although a rise in studies that report morphological changes in sub-regions of the hippocampus, particularly the posterior hippocampus (tail), rather than global differences may also explain

these discrepancies. In order to investigate whether changes in hippocampal volume are related to a state or trait factor for depression, it is necessary to examine whether they are present prior to the onset of depression by analysing neuroimaging data in never-depressed individuals at increased risk for developing depression.

A growing corpus has identified populations at-risk for developing depression with structural abnormalities although findings in the hippocampus have been inconsistent. For example, Chen and colleagues reported reduced grey matter density in bilateral hippocampus of healthy girls at familial risk of developing depression compared to healthy matched controls (Chen, Hamilton, & Gotlib, 2010) and smaller hippocampal volumes were also present in healthy high-risk individuals with a depressed twin (Baare et al., 2010). Conversely, healthy individuals identified as high familial risk for depression that had then developed major depression over a 2 year period showed significant decreases in right amygdala grey matter but not in the hippocampus (Nickson et al., 2016). In young healthy individuals with high neuroticism (another recognised risk factor for depression (Kendler et al., 1993)), no differences in hippocampal volume were observed compared to low neuroticism individuals although surprisingly, older adults with high neuroticism showed increased hippocampal volume which the authors suggested could be a neural marker of resilience to depression (Chan et al., 2016).

In relation to childhood trauma, a smaller left hippocampal volume was reported in women that experienced childhood sexual abuse (Stein, Koverola, Hanna, Torchia, & McClarty, 1997) and also in depressed patients with a history of physical and sexual abuse compared to depressed patients with no history of abuse and healthy controls (Vythilingam et al., 2002). Moreover, childhood maltreatment was also associated with localised reductions in sub-regions of the hippocampus including the CA2-CA3 fields and subiculum (Teicher, Anderson, & Polcari, 2012). However in another study, childhood adversity was not found to be associated with smaller hippocampal volumes (Lenze et al., 2008). In healthy individuals

with a cognitive vulnerability for depression, significant grey matter reductions in the left pre-central gyrus and right fusiform gyrus were observed compared to healthy controls but not in the hippocampus (Zhang et al., 2012). Moreover, healthy women with sub-threshold depression showed no significant volumetric differences in the hippocampus but did show significantly decreased grey matter density (GMD) in the right inferior parietal lobule and increased GMD in the amygdala, PCC and precuneus (Li et al., 2015). GMD refers to the proportion of grey matter in the brain region relative to other tissue types (Mechelli, Price, Friston, & Ashburner, 2005) and is thought to reflect the number and size of neurones and glial cells. Hayakawa and colleagues also reported no significant differences in hippocampal volumes in females with subclinical depression (Hayakawa et al., 2013) although reduced hippocampal volumes have been reported in healthy males with subclinical symptoms (Spalletta, Piras, Caltagirone, & Fagioli, 2014).

In relation to chronotype, previous studies investigating structural differences in healthy individuals are limited. Takeuchi and colleagues (2015) reported that later chronotype (eveningness) was associated with increased rGMD in the precuneus and left PCC (similar to healthy women with subclinical depression (Li et al., 2015)) and reduced rGMD in the bilateral orbito-frontal cortex using VBM analysis, but no differences were reported in the hippocampus using a whole-brain analysis approach (Takeuchi et al., 2015). This finding was recently replicated by Rosenberg and colleagues (2018) who reported increased grey matter volumes in lateral occipital cortex and precuneus in late chronotypes compared to early chronotypes, as well as increased cortical thickness in precuneus and inferior parietal cortex (Rosenberg, Jacobs, Maximov, Reske, & Shah, 2018). Finally, smaller bilateral hippocampal volumes were observed in healthy participants with a late bed time (at 1am or later) compared to participants with medium (11pm or 12pm) and early (9pm or 10pm) bed times (Kuperczko et al., 2015), however chronotype was not explicitly measured in this study.

Taken together, previous evidence suggests that patients with depression show widespread structural abnormalities with the majority of studies reporting hippocampal volume reductions (Cole et al., 2011; Kempton, Salvador, Munafo, et al., 2011; Koolschijn et al., 2009; Videbech & Ravnkilde, 2004) compared to healthy individuals. Other populations recognised as ‘at-risk’ for developing depression also show reductions in hippocampal volume (Baare et al., 2010; Chen et al., 2010; Spalletta et al., 2014; Stein et al., 1997; Teicher et al., 2012; Vythilingam et al., 2002), although findings are inconsistent (Chan et al., 2016; Hayakawa et al., 2013; Lenze et al., 2008; Li et al., 2015; Nickson et al., 2016; Zhang et al., 2012). Two studies have reported structural differences in relation to chronotype (Rosenberg et al., 2018; Takeuchi et al., 2015) but did not use analyses optimised for investigating subcortical structures such as the hippocampus. Another study reported reduced hippocampal volume in healthy individuals with a late bed time (Kuperczko et al., 2015) but did not use an explicit measure of chronotype which is a more complex construct than ‘bed time’, and did not account for sleep quality. Therefore, the first aim of the current study was to replicate the findings of Takeuchi et al (2015) and Rosenberg et al (2018) by exploring whether any differences in GMD in the precuneus/PCC are related to chronotype using VBM with an ROI approach. The *a priori* hypothesis was that earlier chronotype would be associated with reduced GMD in precuneus. In addition, as other at-risk groups show structural abnormalities in widespread regions of the brain, an exploratory whole-brain VBM analysis was conducted and related to chronotype. Secondly, the current weight of evidence suggests other at-risk groups have reduced hippocampal volume, therefore an automated segmentation of the hippocampus was conducted and it was hypothesised that later chronotype would be associated with reduced grey matter volume in bilateral hippocampus, which could reflect a neural vulnerability marker for depression. Finally, regional differences in hippocampal volume using shape analysis have been reported in patients with depression (Maller et al., 2007; Malykhin & Coupland, 2015;

Neumeister et al., 2005; Posener et al., 2003; Watanabe et al., 2017) and healthy individuals that have experienced childhood maltreatment (Teicher et al., 2012). Therefore, it was hypothesised that later chronotype would be associated with localised differences in hippocampal morphology.

## 6.2 Methods

### 6.2.1. Participants

The same participants were used as in chapters 5, 7, 8 and 9 and structural data was acquired for all 50 participants (38 females, age range 18-37 ( $M=21.24$ ,  $SD=3.77$ ). Again, the study was approved by the local ethics committee and written informed consent was obtained prior to any study procedures taking place. Participants were in good physical health and free of concurrent medication. Exclusion criteria were current or previous depression, presence of major depression in a biological parent, diagnosed sleep disorder and contraindication for MR examination. Similarly, chronotype was determined using the rMEQ (Adan & Almirall, 1991b), sleep quality was assayed using the PSQI questionnaire (Buysse et al., 1989) and symptoms of depression and anxiety were measured using the PHQ-4 (Löwe et al., 2010). The time at which the MRI scan took place was between 10am and 5pm.

### 6.2.2. Image data acquisition

Structural MRI data were acquired on a research dedicated 3T Magnetom Trio (Siemens, Erlangen, Germany) fitted with a 32-channel head coil and located at the Combined Universities Brain Imaging Centre (CUBIC). For each participant we collected a three-dimensional  $T_1$ -weighted whole-brain scan (magnetization-prepared rapid acquisition with

gradient echo (MPRAGE), inversion time (TI) = 1100 ms, repetition time (TR) = 1830 ms, echo time (TE) = 3.03 ms, flip angle (FA) = 11°, field of view (FOV) = 256 × 256 × 160 mm<sup>3</sup>, voxel size = 1 × 1 × 1 mm<sup>3</sup>). Participants were asked to remain as still as possible.

### 6.2.3. MRI analysis pipeline

All image analyses were performed using FSL version 5.0.10 (FMRIB Software Library, <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Firstly, structural data were analysed with FSL-VBM (Douaud et al., 2007) <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>, an optimised VBM protocol (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI 152 standard space using non-linear registration (Andersson & Smith, 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Benefits of using a study-specific template for spatial normalisation rather than a standard MNI template are that it accounts for demographic characteristics associated with the study sample and specific scanner non-uniformities (Shen, Sze, & Sterr, 2007). Second, all native grey matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (corresponding to approximately 7 mm FWHM) to reduce noise. The images were then segmented into three tissue types; grey matter, white matter and cerebrospinal fluid (CSF). From this step a partial volume probability map is produced assigning each voxel with a certain tissue class using the hidden Markov random field (HMRF) model (Zhang et al., 2001).



Two masks were created for the *a priori* regions of interest; the hippocampus and the precuneus. The hippocampus masks (left and right) were created using the Harvard-Oxford subcortical structural atlas implemented in FSL. The probability range was set to 75-100% and the mask was applied to the grey matter image from the study specific template. The precuneus mask was spherical and placed over the precuneus/cuneus/superior parietal lobule with a radius of 10 mm and was created using the coordinates of the peak voxel activation ( $x = 7.5$ ,  $y = -73.5$ ,  $z = 24$ ) selected from Takeuchi et al (2015) where chronotype was reported to be associated with regional grey matter differences in this region (Takeuchi et al., 2015). Voxel-wise GLM was then applied and linear effects of chronotype on grey matter volume were tested for significance using non-parametric permutation tests (applying 5000 permutations) using threshold-free cluster enhancement to correct for multiple comparisons across space (Smith & Nichols, 2009). The ‘two-pass’ option was also applied to correct for non-stationarity (lack of uniform smoothness) of the underlying data (Salimi-Khorshidi, Smith, & Nichols, 2011). First, regional differences in GMD of the hippocampus and precuneus were investigated using the ROI masks where age, gender, PSQI score and mood (symptoms of depression and anxiety measured using the PHQ-4) were added in the regression model. Time of scan was not modelled as there is no evidence that timing can influence individual brain structure. Second, an exploratory whole-brain analysis was done using the grey matter image from the study-specific template to investigate whether any other unpredicted differences existed relating to chronotype.

Next, automated segmentation of the hippocampus was performed on the structural data in order to assess the effect of chronotype on hippocampal volume. Segmentation was performed using FSL-FIRST (also included in the FMRIB Software Library) which uses a Bayesian probabilistic approach and is based on shape/appearance models constructed from 336 manually segmented images provided by the Centre for Morphometric Analysis (CMA),

Boston (Patenaude et al., 2011). The manual labels are parameterised as surface meshes which represent volumetric information for each structure. The most probable shape is then ascertained by searching through linear combinations of shape modes of variation based on learned models. The segmentation process also includes boundary correction and registration to a standard MNI152 template at 1mm resolution using 12 degrees of freedom, and to a subcortical mask in order to eliminate voxels outside the subcortical structure. For each subject, the quality of segmentation and registration were manually checked. Volumes of left and right hippocampus, as well as total intracranial volume (TIV) were computed using FIRST and SIENAX in FSL and further analysed using SPSS software. The data were assessed for the assumptions of normality by applying the Shapiro-Wilk test and then partial correlations were performed in order to investigate the impact of chronotype on volume of the hippocampus, controlling for TIV, age, gender, PSQI and mood.

Finally, surface-based vertex analysis was performed to identify localised differences in hippocampal shape which were related to chronotype. This analysis is based on multivariate statistics which creates a 3D mesh of the hippocampus in standard space and performs a univariate test at each vertex of each subject and measures the distance compared to the average hippocampal shape from all subjects. Voxel-wise GLM was applied and linear effects of chronotype on alterations in hippocampal shape were tested for significance using non-parametric permutation tests (again using 5000 permutations and threshold-free cluster enhancement) with the same covariates of no interest included in the regression model (age, gender, PSQI score and mood symptoms).

## 6.3. Results

### 6.3.1. Participants

Participant characteristics are presented in *Table 6* and are the same as those reported in Chapter 5. Measures of anxiety ( $r = 0.13$ ,  $p > .05$ ), depression ( $r = 0.17$ ,  $p > .05$ ) and time of scan ( $r = 0.013$ ,  $p > .05$ ) were not significantly correlated with rMEQ. No systematic bias was observed between the participant's chronotype and the time of day the experiment was performed at as both early and late chronotype were equally likely to attend morning or afternoon/evening sessions. Chronotype scores were similar between male and female participants (independent samples  $t$ -test ( $t(13.73) = -.68$ ,  $p > .05$ ). However, there was a significant correlation between rMEQ and PSQI score ( $r = 0.383$ ,  $p < .01$ ) and age ( $r = -0.334$ ,  $p < .05$ ) such that late chronotype was associated with better sleep quality and older age.

*Table 6. Descriptive statistics.*

<b>Variable</b>	<b><i>M</i> (SD)</b>	<b>Range (this sample)</b>	<b>Range (original scale)</b>
<b>Age</b>	21.24 (3.77)	18-37	-
<b>Gender</b>	Female 38 (76%); Male 12 (24%)	-	-
<b>Sleep quality</b>	7.30 (3.42)	1 - 15	0 – 21
<b>Chronotype</b>	12.62(3.62)	6 - 20	4 – 25
<b>PHQ-4 (Anxiety)</b>	0.91 (1.09)	0 - 5	0 – 6
<b>PHQ-4 (Mood)</b>	1.61 (1.58)	0 - 6	0 -6

*Note.* Data show basic demographics, sleep quality (PSQI), chronotype (rMEQ), mood and anxiety (PHQ-4). Please see Methods for questionnaire details. Values show mean (SD). Also included are the questionnaire range (minimum-maximum) for the study sample and original scale.

### 6.3.2. VBM results

Multiple regression analysis was used to investigate whether there was an association between rGMD and chronotype. Regional GMD was not found to be significantly associated with rMEQ score in the left hippocampus, right hippocampus or precuneus using the ROI approach. Similarly, the exploratory whole-brain analysis revealed no rGMD differences in any brain regions that were associated with chronotype.

### 6.3.3. Segmentation

Right and left hippocampal volumes (normalised to TIV) were similar to previously published findings (Mean = 0.00228, SD = 0.0003, Mean = 0.00226, SD = 0.0003 respectively) (Dean et al., 2016). The Shapiro-Wilk test revealed the data (hippocampal volume) did not meet the assumptions of normality ( $p < .05$ ) so a non-parametric (Spearman's Rho) partial correlation was applied. Hippocampal volume (left and right separately) was not associated with age, sleep quality, mood symptoms or anxiety symptoms when accounting for TIV. A Mann-Whitney U test revealed that left hippocampal volume was marginally (but not significantly) larger in males than females ( $p = .054$ ), but not in right hippocampus ( $p = .066$ ).

Similarly, there was no significant correlation between chronotype and left hippocampal volume  $r_s(41) = .09$ ,  $p > .05$  or right hippocampal volume  $r_s(41) = .13$ ,  $p > .05$  (see *Table 7* and *Figure 17*), controlling for TIV, age, gender, PSQI score and mood.

Table 7. Mean raw hippocampal and TIV volumes across all participants.

Region of Interest (ROI)	Mean volume (mm <sup>3</sup> )	Partial correlation with rMEQ score (p-value)
Left hippocampus	3749.8 (568)	p = .53
Right hippocampus	3782.3 (565)	p = .40
Total intracranial volume (TIV)	1657566.2 (54103)	p = .43

Note. Values show mean (SD). P-values from Spearman's Rho partial correlation with rMEQ score are also displayed where TIV, age, gender, PSQI score and mood were added as covariates.

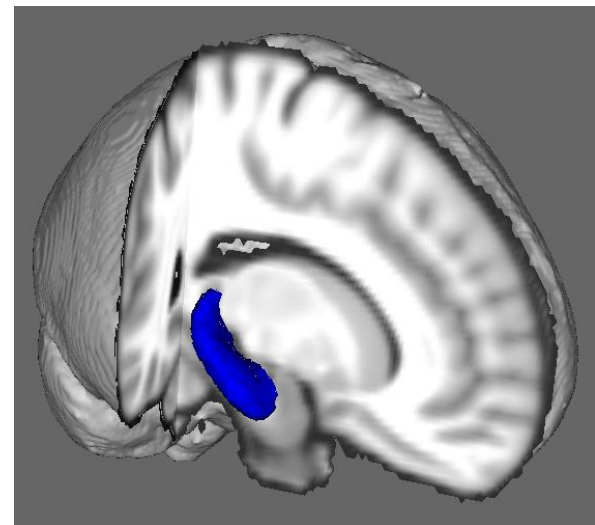
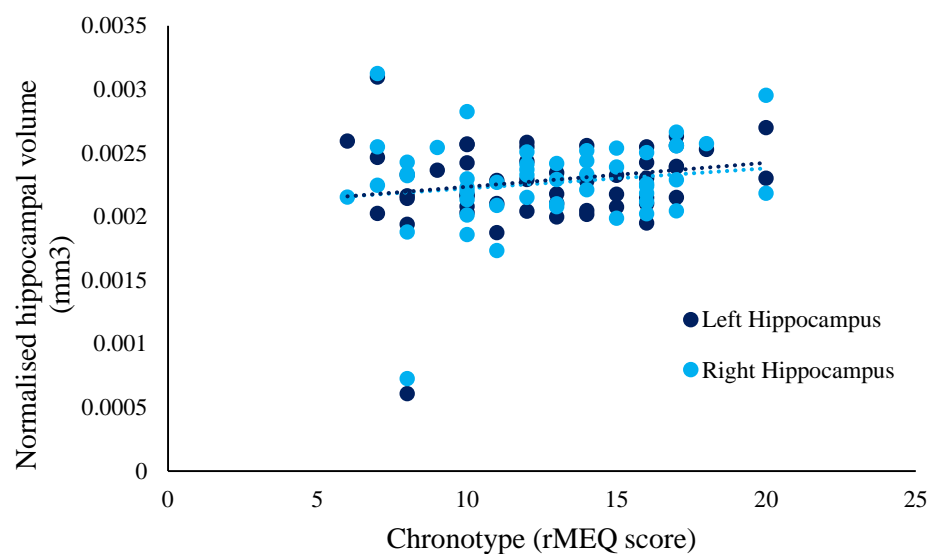


Figure 17. Scatterplot (left) showing non-significant relationship between chronotype and left and right hippocampal volumes (normalised to TIV), and a visual depiction (right) of the segmented right hippocampus overlaid onto a MNI standard 3D rendered brain template.

#### 6.3.4. Vertex analysis

Surface-based vertex analysis was used to investigate whether there was an association between chronotype and regional differences in the shape of the left and right hippocampus. Later chronotype was associated with a trend towards localised atrophy in the subiculum region of the right hippocampus ( $p = 0.054$ ) but not left hippocampus ( $p = 0.403$ ).

### 6.4 Discussion

The current study was a partial replication to explore whether rGMD differences are present in the hippocampus and precuneus in relation to chronotype. This is also the first study, to my best knowledge, to explore hippocampal shape differences and relate these to chronotype. None of the hypotheses made were supported as no significant correlations were observed between chronotype and rGMD in the precuneus or hippocampus regions of interest, or across whole brain. No significant correlation was found between chronotype and right or left segmented hippocampal volume where TIV, age, gender, PSQI score and mood were added as covariates in the model. There was also no relationship between chronotype and regional shape differences in left and right hippocampus although there was a trend towards significance for localised atrophy over the subiculum region of the right hippocampus. Previous evidence has identified late chronotype to be associated with increased prevalence of depression (Antypa et al., 2017; Haraden et al., 2017; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) and reduced hippocampal volume has been reported in depressed patients (Cole et al., 2011; Kempton, Salvador, Munafo, et al., 2011; Koolschijn et al., 2009; Videbech & Ravnkilde, 2004) and some populations at increased risk for depression (Baare et al., 2010; Chen et al., 2010; Spalletta et al., 2014; Stein et al., 1997; Teicher et al., 2012; Vythilingam et al., 2002). Regional differences in hippocampal shape have also been reported

in patients with depression (Malykhin & Coupland, 2015) and healthy individuals that have experienced childhood maltreatment (Teicher et al., 2012). Here, later chronotype was not associated with any anatomical differences in bilateral hippocampus or precuneus.

In contrast to the current findings, a significant decrease in GMD in the PCC/precuneus and OFC was previously associated with earlier chronotype in 776 healthy Japanese participants (Takeuchi et al., 2015). The authors suggested that lower GMD in these regions is a consequence of developmental cortical thinning caused by synaptic pruning, and is associated with matured cognitive abilities and better functioning of these brain areas (Kanai, Dong, Bahrami, & Rees, 2011; Takeuchi et al., 2014; Takeuchi et al., 2011, 2013). This finding was recently replicated in a sample of 48 healthy males (Rosenberg et al., 2018). The precuneus in particular has been associated with self-referential processing and theory of mind (Kjaer, Nowak, & Lou, 2002; Servaas et al., 2013) and reduced GMD in this region has been associated with increased self-discipline and pro-social cognitions (Banissy, Kanai, Walsh, & Rees, 2012; Coutinho, Sampaio, Ferreira, Soares, & Goncalves, 2013). In support of this, Takeuchi and colleagues' finding of reduced GMD in the precuneus associated with morningness was also associated with self-report measures of self-directedness and sympathy (Takeuchi et al., 2015), suggesting the structural differences reported in this region reflect higher competence in these cognitive components. Moreover, healthy women with sub-threshold depression have also been shown to have significantly increased GMD in the precuneus/PCC (Li et al., 2015). The current study was unable to replicate previous findings of altered GMD in the precuneus/PCC. Moreover, an exploratory analysis revealed no other GMD differences associated with chronotype, in contrast to the positive correlation between chronotype and bilateral OFC, and bilateral hypothalamus around the suprachiasmatic nuclei also observed by Takeuchi and colleagues. The difference in findings may be due to the smaller sample size in the current study (50 participants compared to 776) although this was comparable to the sample size reported by

Roesenberg and colleagues (n = 48). Both females and males were also included in the present study whereas Rosenberg and colleagues (2018) only included male participants suggesting structural differences may differ according to gender. However, the current study also included sleep quality (PSQI) as a covariate in the regression model as it has been shown to be an independent factor for current depression and depression severity (Antypa et al., 2016; Muller, Kundermann, & Cabanel, 2016), which could have also accounted for the discrepancies between studies. Taken together, the current findings suggest chronotype is not associated with structural differences in the precuneus or any other brain regions using VBM analysis. However, further large cohort studies with better co-factor analyses are warranted to delineate the impact of chronotype on GMD independent of sleep quality.

The present study also reported no significant differences in hippocampal volume or regional differences in hippocampal shape associated with chronotype although later chronotype was associated with a trend towards localised atrophy over the subiculum in right hippocampus. This finding is in contrast to the majority of studies reporting reduced hippocampal volume (Cole et al., 2011; Kempton, Salvador, Munafo, et al., 2011; Koolschijn et al., 2009; Videbech & Ravnkilde, 2004) and smaller regional differences over the posterior hippocampus in depressed patients (Maller et al., 2007; Malykhin & Coupland, 2015; Neumeister et al., 2005; Posener et al., 2003; Watanabe et al., 2017), with some exceptions (Posener et al., 2003; Videbech & Ravnkilde, 2004). However, the findings are similar to some studies of other at-risk populations that also do not report hippocampal changes (Chan et al., 2016; Hayakawa et al., 2013; Lenze et al., 2008; Li et al., 2015; Nickson et al., 2016; Zhang et al., 2012). In one study, later sleep timing was associated with reduced hippocampal volume when accounting for age and gender (Kuperczko et al., 2015), however this study did not measure chronotype as a complete construct and did not account for sleep quality, limiting the reliability of their findings. The neurotoxicity hypothesis states that reductions in hippocampal



volume are a result of disturbed functioning of the HPA axis which causes an increase in glucocorticoids such as cortisol that damage hippocampal neurones. Therefore, these changes only occur after an individual is diagnosed with depression. Indeed, a number of studies have reported volumetric differences in the hippocampus only in patients with persistent, recurrent depression compared to first-episode patients (McKinnon et al., 2009; Phillips et al., 2015; Sheline et al., 1999). In relation to chronotype, there is limited evidence to suggest late chronotypes have altered levels of stress hormones or disturbed HPA axis functioning (Kudielka et al., 2006; Maierova et al., 2016; Roeser, Meule, Schwerdtle, Kubler, & Schlarb, 2012). The present findings therefore support the neurotoxicity theory (although cortisol was not measured) and suggest changes in hippocampal volume are not present prior to the onset of depression in late chronotype individuals.

Evidence for altered cortisol levels in at-risk populations is mixed, mainly due to methodological considerations and gender differences. However, there is some evidence that healthy individuals with high neuroticism personality trait (Garcia-Banda et al., 2014) and familial risk for depression (Mannie, Harmer, & Cowen, 2007) display hypersecretion of cortisol but previous studies reporting hippocampal differences in these two at-risk populations have been inconsistent (Baare et al., 2010; Chan et al., 2016; Chen et al., 2010; Kendler et al., 1993; Nickson et al., 2016). The most prominent environmental risk factor for depression is childhood abuse and many studies have shown hippocampal reductions in healthy participants (Stein et al., 1997; Teicher et al., 2012) as well as in depressed patients that have experienced childhood trauma compared to patients that have not (Vythilingam et al., 2002). It is thought that early life stress induced by childhood adversity causes neurobiological changes including hippocampal damage and sensitises the individual to stressors occurring later on in life (Hammen, Henry, & Daley, 2000) which can eventually trigger depressive episodes (Heim et al., 2000). For example, healthy women with a history of childhood abuse showed elevated

levels of plasma Adrenocorticotrophic Hormone (ACTH), a hormone secreted by the HPA axis to increase cortisol levels, in response to a psychological stressor compared to both non-abused depressed women and controls (Heim et al., 2000). However, in another study which selected healthy participants and remitted depressed patients with less severe experiences of childhood abuse reported no association with hippocampal volume (Lenze et al., 2008) therefore suggesting only a high level of stress is associated with hippocampal volume reductions prior to the onset of depression. Therefore, it is likely that previous stress, and not risk for depression alone, is a better predictor of hippocampal volume changes prior to the development of depression. Taken together, although late chronotype is recognised as a risk factor for depression, hippocampal volume does not seem to be a neural vulnerability marker for depression in this population. However, future studies investigating the interaction between late chronotype and stress reactivity on hippocampal volume may be useful for exploring risk for depression in this population.

#### 6.4.1. Limitations

A number of limitations should be taken into consideration. The present study excluded participants if they reported a diagnosis of depression in a biological parent but specific genes relating to hippocampal structure were not identified using appropriate genetic analyses. Although we did not report changes in hippocampal structure, this factor is important because it has been shown to be highly genetically determined (Glahn et al., 2005) including specific genetic risk variants associated with hippocampal volume (Baune et al., 2012; Dannlowski et al., 2015; Frodl et al., 2004) and healthy individuals at genetic risk of developing depression have shown hippocampal volume reductions (Baare et al., 2010; Chen et al., 2010). Participants were also not screened for childhood maltreatment which, as discussed above, has been shown to be associated with hippocampal volume although no differences were reported. Finally, there

is some evidence that excessive alcohol use is associated with reduced hippocampal volume (Wilson, Bair, Thomas, & Iacono, 2017) and Internet Gaming Disorder (IGD) is associated with larger volumes in the hippocampus/amygdala and precuneus compared to healthy controls (Yoon et al., 2017). Whilst these associations have been reported in participants with a clinical diagnosis and the present study aimed to recruit participants in good health, participants with these disorder were not explicitly screened for and future studies should account for this.

## 6.5. Conclusion

In conclusion, no association was found between late chronotype and GMD in the hippocampus, precuneus or any other brain regions. Although late chronotype is a recognised risk factor for depression, the current findings suggest alterations in brain structure do not represent a neural vulnerability marker in this population, similar to studies of some other at-risk groups. This supports the neurotoxicity theory of depression although it is likely that a combination of factors such as genetics, childhood trauma and stress contribute towards structural differences associated with the aetiology of depression as well.

## **CHAPTER SEVEN**

# **Altered resting-state connectivity and the Default Mode Network**

### **7.1. Introduction**

As reported in Chapter 4, resting-state fMRI studies of major depression have reported altered connectivity in a number of resting-state networks. In particular, converging evidence that suggests depression is associated with disruption to the Default Mode Network (DMN). This network is preferentially activated when the participant is internally focused; for example, during daydreaming, future planning, memory retrieval and thinking from different perspectives (Raichle et al., 2001). A number of brain regions have been consistently associated with the DMN. These include the posterior cingulate cortex (PCC)/precuneus, medial prefrontal cortex (mPFC), ventral anterior cingulate cortex (ACC), and inferolateral parietal cortex (Dutta et al., 2015). The literature describes a dissociation between the anterior (centred on the mPFC) and posterior regions (centred on the PCC and inferolateral parietal lobes) of the DMN (Zhou et al., 2010). The anterior DMN is implicated in self-referential processing and emotion regulation whereas the posterior DMN is implicated in consciousness and memory processing (Zhu et al., 2012). A pattern of altered connectivity within the DMN has been identified in depressed individuals. However, the directionality and extent of this altered connectivity is unclear as both increases and decreases have been reported across a broad range of brain regions (Brakowski et al., 2017; Broyd et al., 2009; Dutta et al., 2015; Greicius, 2008; Zhang et al., 2016). These inconsistencies might be related to differences in the depressed population

studied; for example, age, medication status, pharmacotherapy and depression severity. By studying patients with depression, it is also difficult to determine whether altered connectivity arises as a result of the disorder or pre-exists development of the disorder. Therefore, it is necessary to examine DMN connectivity in healthy, never-depressed individuals at increased risk for depression.

A growing corpus has identified populations at-risk for developing depression with resting-state connectivity abnormalities within the DMN. For example, Servaas *et al.*, reported high neuroticism (a recognised risk factor for depression) was associated with reduced functional connectivity between an occipito-parietal seed region (including the PCC, precuneus and cuneus) and middle cingulate gyrus, insula, and postcentral gyrus, and increased connectivity between the same region and the calcarine sulcus, lingual gyrus and inferior frontal gyrus following exposure to self-referential criticism (Servaas *et al.*, 2013). Reduced connectivity within the DMN has also been reported in children at familial risk of depression (i.e. children that have a 1<sup>st</sup> degree relative with depression) (Bellgowan *et al.*, 2015) although hyperconnectivity of the DMN has also been reported in this at-risk group (Chai *et al.*, 2016; Posner *et al.*, 2016). Two of these studies (Chai *et al.*, 2016; Posner *et al.*, 2016) also reported a reduced anticorrelation between the DMN and the central executive network (CEN) including the dorso-lateral prefrontal cortex (DLPFC) suggesting an impairment in attentional control or impulsivity within these groups (Chai *et al.*, 2016; Posner *et al.*, 2016). Healthy participants that have experienced childhood trauma, another recognised risk factor for depression, also show altered DMN connectivity (Lu *et al.*, 2017).

Additionally, a number of studies have identified altered DMN connectivity in healthy individuals with subclinical symptoms of depression. Rzepa and McCabe reported decreased functional connectivity between the amygdala and PCC/precuneus as well as between the DMPFC and precuneus in adolescents with high scores on the mood and feelings questionnaire

(MFQ) compared to age-matched participants scoring low on the same instrument (Rzepa & McCabe, 2016). Zhang et al., reported that individuals with a cognitive vulnerability for depression (a negative cognitive style where the individual attributes negative events to external causes that are beyond their control) had reduced ALFFs in bilateral orbitofrontal cortex and increased ALFF in insula cortex and left fusiform gyrus, similar to differences observed in the depressed patient group (Zhang et al., 2016). Similarly, reduced fractional ALFF (fALFF) in bilateral precuneus and left posterior cerebellum was associated with higher nonclinical depressive symptomology in young adults (Wei et al., 2015). Taken together, the data indicate that healthy participants with sub-threshold symptoms of depression show altered functional connectivity within the DMN, similar to other at-risk groups (Bellgowan et al., 2015; Chai et al., 2016; Lu et al., 2017; Posner et al., 2016; Servaas et al., 2013), which may represent a neural vulnerability marker prior to the development of depression.

In sum, current evidence suggests that patients with depression have altered resting-state functional connectivity within the DMN (Brakowski et al., 2017; Broyd et al., 2009; Dutta et al., 2015; Greicius, 2008; Zhang et al., 2016), compared to healthy individuals. Other healthy populations recognised as ‘at-risk’ for developing depression also show altered resting-state DMN connectivity (Bellgowan et al., 2015; Chai et al., 2016; Lu et al., 2017; Posner et al., 2016; Rzepa & McCabe, 2016; Servaas et al., 2013; Wei et al., 2015; Zhang et al., 2016). However, to my knowledge, resting-state functional connectivity has not been investigated in relation to chronotype. Here, a sample of healthy individuals were recruited with a range of chronotypes that were free of current or previous diagnosis of depression, family history of depression or sleep disorder to undergo rsfMRI. It was hypothesised that later chronotype would be associated with reduced functional connectivity within the DMN similar to the pattern of activity observed in depressed patients and other at risk groups.

## 7.2. Methods

### 7.2.1. Participants

The same participants were used as in chapters 5, 6 and 8 but resting-state data was acquired for 46 participants due to time restrictions (34 females, age range 18-37 ( $M = 21$ ,  $SD = 3.2$ ). Again, the study was approved by the local ethics committee and written informed consent was obtained prior to any study procedures taking place. Participants were in good physical health and free of concurrent medication. Exclusion criteria were current or previous depression, presence of major depression in a biological parent, diagnosed sleep disorder diagnosed sleep disorder (also a recognised risk factor for depression (Morphy, Dunn, Lewis, Boardman, & Croft, 2007)) and contraindication for MR examination. All criteria were assessed via self-report. Similarly, chronotype was determined using the rMEQ (Adan & Almirall, 1991a), sleep quality was assayed using the PSQI questionnaire (Buysse et al., 1989) and symptoms of depression and anxiety were measured using the PHQ-4 (Löwe et al., 2010). The time at which the MRI scan took place was between 10am and 5pm.

### 7.2.2. Image data acquisition

All imaging data were acquired on a research dedicated 3T Magnetom TIM Trio (Siemens, Erlangen, Germany) fitted with a 32-channel head coil and located at the Combined Universities Brain Imaging Centre (CUBIC). For each participant, a  $T_1$ -weighted whole-brain scan (magnetization-prepared rapid acquisition with gradient echo (MPRAGE)) was collected, inversion time ( $T_1$ ) = 1100 ms, repetition time ( $T_R$ ) = 1830 ms, echo time ( $T_E$ ) = 3.03 ms, flip angle (FA) =  $11^\circ$ , field of view (FOV) =  $256 \times 256 \times 160 \text{ mm}^3$ , voxel size =  $1 \times 1 \times 1 \text{ mm}^3$ . Functional MR data were acquired using a  $T_2^*$ -weighted echo planar imaging sequence (EPI,  $T_R$  = 3000 ms,  $T_E$  = 31 ms, FA =  $85^\circ$ , FOV =  $192 \times 192 \times 126 \text{ mm}^3$  [42 slices, voxel

size =  $3 \times 3 \times 3 \text{ mm}^3$ ], number of measurements = 200, imaging bandwidth = 752 Hz/px, GRAPPA acceleration factor = 2). Gradient echo field mapping data were also acquired for EPI off-resonance distortion correction ( $T_R = 400 \text{ ms}$ ,  $T_{E1} = 5.19 \text{ ms}$ ,  $T_{E2} = 7.65 \text{ ms}$ , flip angle =  $60^\circ$ , FOV =  $192 \times 192 \times 126 \text{ mm}^3$ , voxel size =  $3 \times 3 \times 3 \text{ mm}^3$ ). The resting-state scan lasted approximately 10 minutes and participants were instructed to remain awake with their eyes open.

### 7.2.3. FMRI analysis pipeline

All image pre-processing and analyses were performed using FSL version 5.0.10 (FMRIB Software Library, <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). The following pre-statistical processes were applied to all fMRI data: non-brain removal; rigid-body motion correction; high-pass temporal filtering (Gaussian-weighted least-squares fitting with frequency cut-off point = 60 s); correction of off-resonance geometric distortions in the EPI data using  $B_0$  field maps derived from the dual-echo gradient echo dataset; artifact removal based on probabilistic ICA (Independent Component Analysis – see below); spatial normalization to Montreal Neurological Institute (MNI152) 2 mm isotropic atlas space using boundary-based registration and non-linear registration (during registration, signal loss, resulting from through-slice field gradients, was calculated and used as a cost function mask to exclude voxels where signal loss was greatest); Gaussian filtering (full width at half maximum (FWHM) = 5 mm).

### 7.2.4. Controlling for structured noise

Manual ICA-based artifact removal was conducted where the independent component maps for each participant were visually inspected to identify noise components based on both the spatial layout of the component maps and the power spectra of the associated time series



(Griffanti et al., 2016). Components with variance labelled as noise were subsequently regressed from each individual's data prior to statistical modelling (see below).

#### 7.2.5. Analysis of rsfMRI data

Dual regression (Filippini et al., 2009) was used to derive subject-specific DMN maps based on a well validated template (Smith et al., 2009). In the first step, the DMN template was included as a spatial regressor in a General Linear Model (GLM) against each individual's rsfMRI denoised data (as described above). The output from this analysis were included in a second GLM (now as temporal regressors), again against the pre-processed rsfMRI data to estimate subject-specific statistical parametric maps where the parameter estimates at each voxel represent connectivity strength with the DMN.

At the group level, linear effects of chronotype on DMN connectivity were tested for significance using non-parametric permutation tests (applying 5000 permutations). As previous work has reported an association between lower regional grey matter density and late chronotype (Takeuchi et al., 2015), grey-matter probability maps were included as a voxel-wise anatomical covariate. Briefly, each individual subject's T<sub>1</sub>-weighted high-resolution anatomic image was registered to a standard template [Montreal Neurological Institute (MNI) 152 stereotactic template] using an affine procedure with a 12-parameter fit (Jenkinson & Smith, 2001). The MRI images were then segmented into three tissue classes (cerebrospinal fluid, white matter and grey matter) (Zhang et al., 2001). Grey-matter probability maps were masked (i.e. non-brain voxels set to zero) and smoothed to yield images with similar smoothness to the corresponding functional data, in order to minimize partial volume effects (Oakes et al., 2007). Lastly, the resulting smoothed grey-matter probability maps were demeaned prior to inclusion

in the group analysis. Control of the family-wise error rate was obtained using threshold-free cluster enhancement ( $p < 0.05$ ).

## 7.3. Results

### 7.3.1. Participants

Participant characteristics are presented in *Table 8*. Inspection of the behavioural data revealed that participants with a wide range of chronotypes (6 – 20) were recruited. Measures of anxiety ( $r_s(46) = 0.15$ ,  $p = .33$ ), depression ( $r_s(46) = 0.17$ ,  $p = .25$ ) and time of scan ( $r_s(46) = 0.05$ ,  $p = .74$ ) were not significantly correlated with rMEQ. Chronotype scores were similar between male and female participants (independent samples *t*-test ( $t(13.96) = .46$ ,  $p = .65$ ). However, there was a significant correlation between rMEQ and PSQI score ( $r(46) = 0.35$ ,  $p = 0.016$ ) and age ( $r(46) = -0.39$ ,  $p = .007$ ) such that late chronotype was associated with better sleep quality and older age.

Table 8. Descriptive statistics and basic demographics.

Variable	M (SD)	Range (this sample)	Range (original scale)
Age	21.04 (3.21)	18-37	-
Gender	Female 34 (74%); Male 12 (26%)	-	-
Sleep quality	7.50 (3.49)	1 - 15	0 – 21
Chronotype	12.89(3.62)	6 - 20	4 – 25
PHQ-4 (Anxiety)	0.91 (1.13)	0 - 5	0 – 6
PHQ-4 (Mood)	1.65 (1.57)	0 - 6	0 -6

*Note.* Values show mean (SD). Also included are the questionnaire range (minimum-maximum) for the study sample and original scale. Sleep quality (PSQI = Pittsburgh Sleep Quality Index), chronotype (rMEQ = reduced Morningness Eveningness Questionnaire), mood and anxiety (PHQ-4 = Patient Health Questionnaire).

### 7.3.2. Resting-state connectivity

Firstly, the DMN was identified across all participants (see *Figure 18*). A whole-brain analysis was then conducted including PSQI score, gender, age, mood and anxiety levels (computed as the sum of the mood and anxiety components of the PHQ-4), time of scan and voxel-wise grey-matter probability maps as covariates of no interest. A significant positive correlation was observed between chronotype and functional connectivity within nodes of the DMN, including; bilateral PCC and precuneus (MNI coordinates of peak voxel: x = 10, y = -54, z = 38, t = 4.24, cluster volume 32 mm<sup>3</sup>, p = 0.0496, see *Figure 19*), such that participants with lower rMEQ scores (increased late chronotype) showed decreased connectivity within these regions. There were no regions that exhibited a negative association between chronotype and functional connectivity within the DMN.

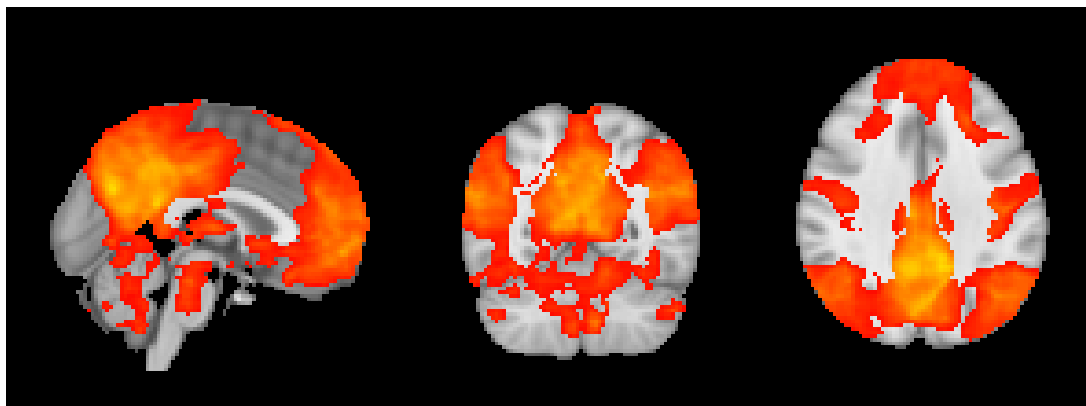


Figure 18. The Default Mode Network (DMN) identified across all 46 participants using Independent Component Analysis (ICA) matched onto a template.

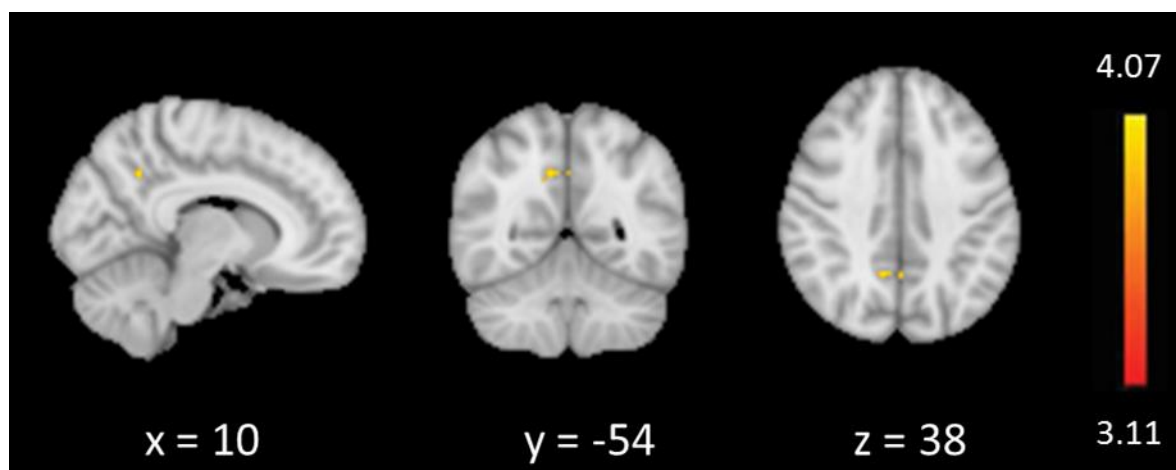


Figure 19. Brain regions identified as showing functional connectivity within the DMN that are significantly positively correlated with chronotype, overlaid onto a MNI standard brain. Lower numerals refer to coordinates in Montreal Neurological Institute (MNI) space. Colour bar and numerals:  $t$  value range.

## 7.4. Discussion

To my knowledge, this is the first study to explore resting-state connectivity of the DMN and relate this to chronotype. A significant positive correlation was observed between functional connectivity within the PCC/precuneus and rMEQ score at rest, such that later chronotype (lower rMEQ score) was associated with reduced connectivity within these nodes of the DMN. This effect was found in subjects with no previous diagnosis of depression or sleep disorder, and was not driven by sleep quality, age, gender, measures of mood and anxiety, time of scan or cortical grey matter volume. Interestingly, two previous studies have reported that later chronotype is associated with increases in precuneus GMD and cortical thickness (Rosenberg et al., 2018; Takeuchi et al., 2015) but this was not replicated in the present investigations (Chapter 6) suggesting changes in brain structure are not underlying the altered resting-state connectivity in precuneus observed here. Previous research has identified late chronotype to be associated with increased risk for depression (Antypa et al., 2016; Au & Reece, 2017; Haraden et al., 2017; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) and altered DMN connectivity has been reported in depressed patients (Brakowski et al., 2017; Broyd et al., 2009; Dutta et al., 2015; Greicius, 2008; Zhang et al., 2016) and individuals at increased risk for depression (Bellgowan et al., 2015; Chai et al., 2016; Lu et al., 2017; Norbury, Mannie, & Cowen, 2011; Posner et al., 2016; Rzepa & McCabe, 2016; Servaas et al., 2013; Wei et al., 2015; Zhang et al., 2016). Here, it was observed that later chronotype was associated with reduced DMN functional connectivity which may reflect a neural mechanism underlying vulnerability to depression in late chronotype individuals.

The current finding is consistent with a number of studies reporting reduced connectivity within the DMN of depressed patients. For example, Zhu et al., reported reduced resting state connectivity in PCC/precuneus in first episode, treatment-naïve patients (Zhu et

al., 2012), and Liu et al., reported decreased fALFF in right precuneus in recurrent MDD patients compared to remitted patients and healthy controls (Liu et al., 2017). However, others have reported enhanced connectivity within the precuneus and other brain regions related to the DMN (Greicius et al., 2007; Sheline et al., 2010). The discrepancies between studies could be due to differences between samples of depressed patients. It has been reported that patients that develop depression early in life (before 30 years) display significantly increased ReHo in left precuneus compared to patients with later adult onset depression (Shen et al., 2017), and recurrent depressed patients had reduced fALFF in the precuneus compared to both remitted patients and healthy controls, and this was correlated with the number of depressive episodes (Liu et al., 2017). Further, altered connectivity within the precuneus has been reported to normalise after 12 weeks of antidepressant medication in patients with MDD (Li et al., 2013) and differences in connectivity between sub-regions of the precuneus may exist (Zhu, Lin, Lin, Zhuo, & Yu, 2018). Therefore, differences in the directionality of DMN connectivity in depression may reflect the heterogeneous nature of the disorder as well as differences in age, medication status, pharmacotherapy, depression severity, number of episodes and different analysis methods (see-based, ICA, ALFF, ReHo) making findings difficult to compare between studies.

More apposite to the current research, reduced DMN functional connectivity has also been observed in some studies of healthy individuals at increased risk for depression. For example, reduced connectivity within the PCC/precuneus has been reported in individuals with high neuroticism (Servaas et al., 2013), children at familial risk of depression (Bellgowan et al., 2015), adolescents with low mood scores (Rzepa & McCabe, 2016) and has been associated with young adults with nonclinical depressive symptomology (Wei et al., 2015). More generally, at-risk groups also have displayed decreased connectivity in other nodes of the DMN (Lu et al., 2017; Zhang et al., 2016) and a meta-analysis revealed that first episode treatment-

naïve depressed patients generally displayed decreased brain activity in a number of brain regions including the precuneus and PCC (Zhong, Pu, & Yao, 2016). It may therefore be that reduced functional connectivity in the PCC/precuneus of late chronotypes represents a neural vulnerability marker, similar to treatment-naïve patients with first-episode depression and other at-risk groups, which could underlie their increased risk for the disorder. Three other studies of children at familial risk of depression report opposite findings, including increased connectivity in the PCC/precuneus (Norbury et al., 2011; Posner et al., 2016) and other nodes of the DMN (e.g. subgenual ACC and orbitofrontal cortex) (Chai et al., 2016; Norbury et al., 2011). These findings could again reflect differences in samples used between studies. Alternatively, it is likely that children with familial risk have a different neural signature associated with risk for depression compared to late chronotypes which may also explain the conflicting findings.

The DMN overlaps with regions involved in social cognition e.g. theory of mind, internalising thoughts and recalling autobiographic memories (Leech & Sharp, 2014; Mars et al., 2012). In particular, the precuneus has been associated with self-referential processing and theory of mind. For example, using Positron Emission Tomography (PET), Kjaer et al., (2002) reported recruitment of the precuneus when participants were reflecting on their own personality traits compared to personality traits of a neutral third person (Kjaer et al., 2002). In individuals with high neuroticism, a reduction in resting state functional connectivity within the precuneus was observed after participants were criticised for not remaining still during their MRI scan (Servaas et al., 2013). It may be therefore, that the reduction in precuneus connectivity within the DMN observed in the current data reflects self-critical thoughts in late chronotype individuals although this was not measured directly. Indeed, differences in recall and recognition of self-referential personality trait words has been associated with chronotype. For example, in Chapters 2 and 3 (and Berdynaj et al., 2016) late chronotypes took longer to correctly recognise self-referential agreeable vs. disagreeable personality trait words than early-

intermediate chronotypes and that late chronotype was associated with greater recall accuracy of disagreeable words. Similar findings have also been reported in individuals with high neuroticism (Chan et al., 2007). Interestingly, late chronotypes have also been reported to better recognise, interpret and understand oneself and other's emotions as compared to early chronotypes (Stolarski & Jankowski, 2015) which the authors suggest might predispose late chronotypes to low mood and negative affective states, and is consistent with evidence suggesting late chronotypes are at increased risk of developing depression (Antypa et al., 2016; Au & Reece, 2017; Haraden et al., 2017; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013). The ability to understand and represent the beliefs of others, a specific component of 'theory of mind', has been shown to recruit the PCC/precuneus (Fletcher et al., 1995; Rilling, Sanfey, Aronson, Nystrom, & Cohen, 2004; Saxe & Powell, 2006). It could be that the ability to better interpret the beliefs of others contributes to self-critical thoughts observed in late chronotypes, mediated by the PCC/precuneus.

Alternatively, evidence also implicates the PCC in attentional processing. However, Leech et al., (2011) reported a dissociation between dorsal and ventral regions of the PCC during an attentionally demanding N-back working memory task, despite reductions in overall activation. As task difficulty increased, the ventral PCC showed reduced integration with the DMN and reduced anticorrelation with the cognitive control network (CCN) whereas the dorsal PCC (adjacent to the precuneus) showed increased DMN integration and greater anticorrelation with the CCN. The dorsal PCC also displays functional connectivity with both the DMN and attentional networks at rest which suggests the role of the dorsal PCC may also be to allocate attention between the DMN and CCN (Leech, Kamourieh, Beckmann, & Sharp, 2011). Liu and colleagues suggested that the significantly decreased fALFF in the right precuneus of patients with recurrent depression may reflect reduced attentional control capacity (Liu et al., 2017). Therefore, it may be that the reduction in resting-state connectivity in the PCC/precuneus



observed here in late chronotypes also represents a reduced ability to allocate attentional resources between internal and external sources, similar to other at-risk groups too (Chai et al., 2016; Posner et al., 2016). Taken together, late chronotype is associated with altered functional connectivity within the PCC/precuneus of the DMN, similar to patients with depression and other at-risk groups, which may in part explain their increased risk of developing MDD. This finding could reflect a neural marker of reduced allocation of attentional resources at rest, or an increase in self-critical thoughts contributed by a better understanding of other people's beliefs. However, future studies designed to directly manipulate these variables are warranted.

The underlying mechanisms linking chronotype to altered DMN connectivity are unclear. However, a recent study by Hodkinson and colleagues (2014) reported diurnal variations in rsfMRI connectivity and regional cerebral blood flow (rCBF) in the DMN of healthy volunteers (Hodkinson et al., 2014) suggesting a circadian control mechanism for this network. Connectivity within the DMN has been shown to be associated with a number of genes responsible for ion channels, vesicle trafficking, cytoskeleton remodelling and are therefore implicated in regulating neurotransmitter release and recycling (Wang et al., 2015). It could be therefore, that circadian clock genes (that generate endogenous cell rhythmicity via feedback loops) have overlapping functions in regulating neurotransmission. For example, there is evidence that dopamine, an important neurotransmitter in the reward circuit and implicated in depression, displays diurnal fluctuations in the nucleus accumbens (Hampp et al., 2008; Hood et al., 2010), suggesting the circadian clock influences the entire reward network. Animal studies also show that clock genes are expressed in the mood regulation circuit including in the ventral tegmental area, prefrontal cortex and amygdala, which is known to be disrupted in depression and in healthy individuals with a later chronotype (as reported in Chapter 5). Mood disorders have also been associated with polymorphisms in certain circadian clock genes (Albrecht, 2013). For example, BMAL1 and PER3 genes have been associated with bipolar

disorder (Nievergelt et al., 2006), PER2, NPAS2 and BMAL1 genes have been associated with increased risk for seasonal affective disorder (Partonen et al., 2007) and the Cry2 gene has been implicated in vulnerability for depression (Lavebratt et al., 2010). Interestingly, a single nucleotide polymorphism (SNP) in the CLOCK gene (T3111C) has been associated with a higher recurrence rate of bipolar depression (Benedetti et al., 2003) and in some studies, late chronotype (Katzenberg et al., 1998; Mishima, Tozawa, Satoh, Saitoh, & Mishima, 2005). Taken together, it is speculated that altered functional connectivity within the DMN observed in the present study could be related to overlapping functions between genes that regulate neurotransmission and circadian rhythms.

#### 7.4.1. Specific limitations

A number of specific limitations associated with the current methodology should be taken into consideration. Firstly, wakefulness was not monitored during scanning (e.g. using an eye-tracker) and therefore an effect of microsleeps on the current results cannot be excluded. However, the participants were instructed to remain awake with their eyes open during the scan. Future studies would benefit from additional measures of arousal using eye-tracking technology or physiological monitoring to assure participant compliance. Secondly, a blood assay was not conducted and so the neuroendocrine effects on the current results cannot be ruled out although the effect of hormones on resting state connectivity is unclear.

## 7.5. Conclusion

A clear association was found between late chronotype and reduced functional connectivity of the PCC/precuneus within the DMN at rest. This finding suggests that late chronotype is associated with an altered neural signature similar to that seen in depressed individuals and other at-risk groups and which could underlie vulnerability for the disorder.

# CHAPTER EIGHT

## Exploring changes in neurometabolites using <sup>1</sup>H-MRS

### 8.1. Introduction

As reported in Chapter 4, proton MRS studies of major depression have identified changes in a number of neurometabolites across the brain. In particular, an imbalance of excitatory and inhibitory neurotransmission has been implicated in the pathophysiology of depression and abnormalities in glutamate and GABA concentrations have been widely reported in patients with depression using proton MRS (Lener et al., 2017). Some attention has also been given to the role of NAA in depression as this neurometabolite is thought to represent neuronal viability and some studies show it is decreased in depression (Bhagwagar et al., 2007; Gruber et al., 2003; Mu et al., 2007; Sözeri-Varma et al., 2013; Vythilingam et al., 2003). However, studies of glutamate, GABA and NAA in depression have largely produced discrepant findings, most likely due to sensitivity to medication status and symptom severity as well as methodological issues associated with MRS (e.g. poor quality spectra, overlapping signals, different analysis packages). The most replicated finding is an overall reduction in GABA in the occipital cortex of depressed patients compared to healthy controls (Price et al., 2009; Sanacora et al., 2004; Sanacora et al., 1999). However, it is still unclear whether changes in neurometabolites such as GABA occur as a result of depression or are present prior to the onset of the disorder. To address this, it is therefore necessary to examine neurometabolite levels in never-depressed individuals at increased risk for developing depression.

There is a limited amount of research investigating changes in neurometabolites levels in at-risk populations. In relation to neuroticism, Ryman and colleagues reported that an increase in NAA levels in the right parietal cortex significantly predicted high neuroticism trait in healthy participants using a biochemical model and stepwise regression in a cross-sectional design (Ryman et al., 2011). Whereas, changes in GABA levels in the frontal lobes are not thought to be related to neuroticism but to extraversion instead (Goto et al., 2010). In healthy individuals with a family history of depression, elevated levels of glutamate, but no changes in GABA, have been reported in the occipital cortex (Taylor, Mannie, Norbury, Near, & Cowen, 2011) and increased levels of Glx have also been reported in the hippocampus (Mannie et al., 2014). However, this study (Mannie et al, 2014) measured a composite measure of glutamate and glutamine (Glx) from a brain region which is known to be difficult to obtain good quality spectra from. This methodology limits the findings because specific changes in glutamate could not be reliably identified, and the large MRS voxel used could have included contributions from neighbouring regions such as the amygdala. Finally, experience of childhood emotional abuse was found to be negatively associated with NAA, Cr and Cho levels in three regions of interest; the rostral PFC, premotor cortex and secondary sensorimotor cortex, in healthy controls (Raparia et al., 2016). Interestingly, this association was not present in patients with generalised anxiety disorder in the same study.

Taken together, previous evidence suggests widespread changes in neurometabolites (including glutamate, GABA and NAA) in depression although findings are equivocal which most likely reflects studies that suffer from small sample sizes and a lack of replication. Other populations recognised as ‘at-risk’ for developing depression have also shown changes in these neurometabolites although findings are limited. To my knowledge, however, no one has yet investigated this in relation to chronotype. Therefore, the aim was to explore whether differences in glutamate, GABA and NAA in the occipital cortex (representing a global

measure) are related to chronotype using single-voxel <sup>1</sup>H-MRS. The most replicated finding in depression is a reduction in GABA in the occipital cortex compared to controls (Romeo et al., 2018), so it was hypothesised that late chronotype would be associated with reduced levels of GABA that could represent a vulnerability marker for depression in these individuals. Limited studies have also reported increased levels of glutamate and reduced levels of NAA in the occipital cortex in patients with depression (Bhagwagar et al., 2007; Sanacora et al., 2004) and in healthy individuals with an increased familial risk for depression (Taylor et al., 2011). Therefore, the guarded prediction was made that late chronotype would be associated with increased glutamate and reduced NAA.

## 8.2. Methods

### 8.2.1. Participants

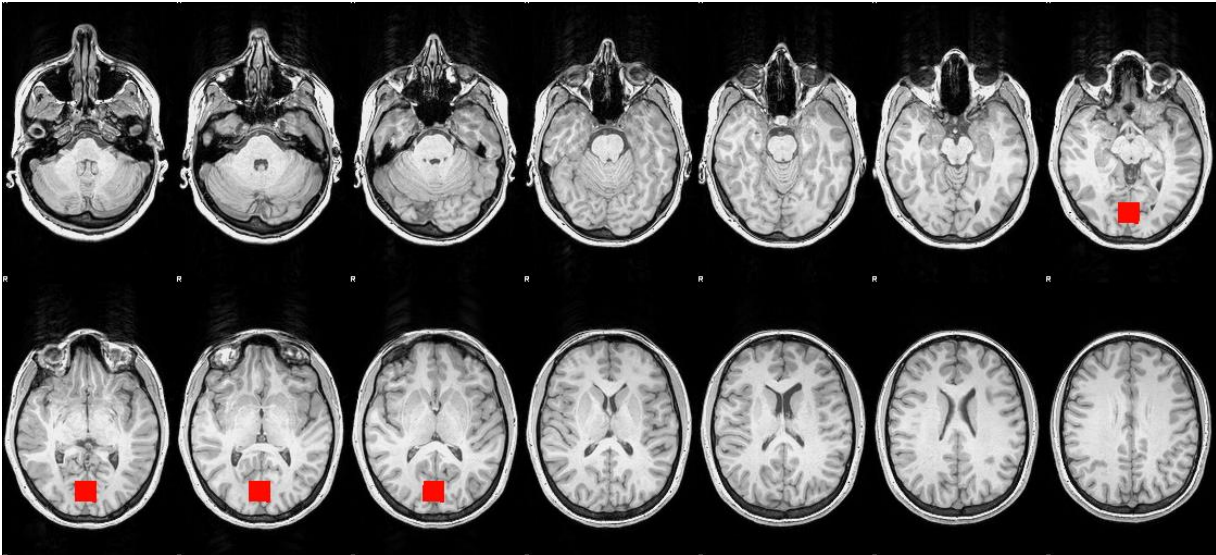
The same participants were used as in chapters 5, 6 and 7 but data was acquired for a subset of 40 participants (30 females, age range 18-37,  $M = 21.65$ ,  $SD = 4.09$ ) due to time constraints. The study was approved by the local ethics committee and written informed consent was obtained prior to any study procedures taking place. Participants were in good physical health and free of concurrent medication. Exclusion criteria were current or previous depression, presence of major depression in a biological parent, diagnosed sleep disorder and contraindication for MR examination. Similarly, chronotype was determined using the rMEQ (Adan & Almirall, 1991a), sleep quality was assayed using the PSQI questionnaire (Buysse et al., 1989) and symptoms of depression and anxiety were measured using the PHQ-4 (Löwe et al., 2010). The time at which the MRI scan took place was between 10am and 5pm.

### 8.2.2. Image data acquisition

All imaging data were acquired on a research dedicated 3T Magnetom TIM Trio (Siemens, Erlangen, Germany) fitted with a 32-channel head coil and located at the Combined Universities Brain Imaging Centre (CUBIC). For each participant a  $T_1$ -weighted whole-brain scan (magnetization-prepared rapid acquisition with gradient echo (MPRAGE) was collected, inversion time ( $T_1$ ) = 1100 ms, repetition time ( $T_R$ ) = 1830 ms, echo time ( $T_E$ ) = 3.03 ms, flip angle (FA) =  $11^\circ$ , field of view (FOV) =  $256 \times 256 \times 160 \text{ mm}^3$ , voxel size =  $1 \times 1 \times 1 \text{ mm}^3$ ). The  $T_1$ -weighted gradient echo image for each participant was used as a reference scan to plan voxel placement.

### 8.2.3. Single voxel spectroscopy

Non-invasive measures of a range of metabolites were acquired from a single voxel with  $^1\text{H}$  MRS using the SPECIAL (SPin ECho full Intensity Acquired Localised spectroscopy) sequence (Mekle et al., 2009; Near et al., 2013) with water suppression ( $T_R$  = 3200 ms,  $T_E$  = 8.5 ms, 128 averages). A short-TE sequence (SPECIAL) was chosen because of its utility to detect a large number of metabolites with high precision and without the need for spectral editing, and for its reliable quantification of glutamate (Schubert, Kuhn, Gallinat, Mekle, & Ittermann, 2017). Automatic shimming of the  $B_0$  magnetic field was performed before each acquisition by optimising the linear X, Y and Z gradients to improve the field of homogeneity. The MRS voxel of interest had dimensions of  $20 \times 20 \times 20 \text{ mm}$  and was manually placed medially in the occipital cortex for each individual, and outer volume suppression was applied. An example placement of the voxel of interest can be seen in *Figure 20*. Four water-unsuppressed spectra were also acquired. The acquisition protocol lasted approximately five minutes and participants were asked to remain as still as possible.



*Figure 20. Example of voxel placement in occipital cortex planned onto the individual's  $T_1$ -weighted scan using axial view.*

#### 8.2.4. Quality control

Spectra were processed semi-automatically by removing motion-corrupted averages and correcting for frequency and phase drift before signal averaging (Simpson, Devenyi, Jezzard, Hennessy, & Near, 2017). The line width, signal to noise ratio (SNR) and baseline of each spectrum were checked to ensure the robustness of the data and were generally considered to be reliable if spectra had a FWHM  $< 0.150$  ppm and SNR  $> 50$ . Additionally, spectra with a Cramer-Rao lower bound (CRLB) greater than 20% were considered to be of poor quality. All participants' data met these quality control measures and were included in further analyses. An example of a spectrum acquired during data collection is presented in *Figure 21*.



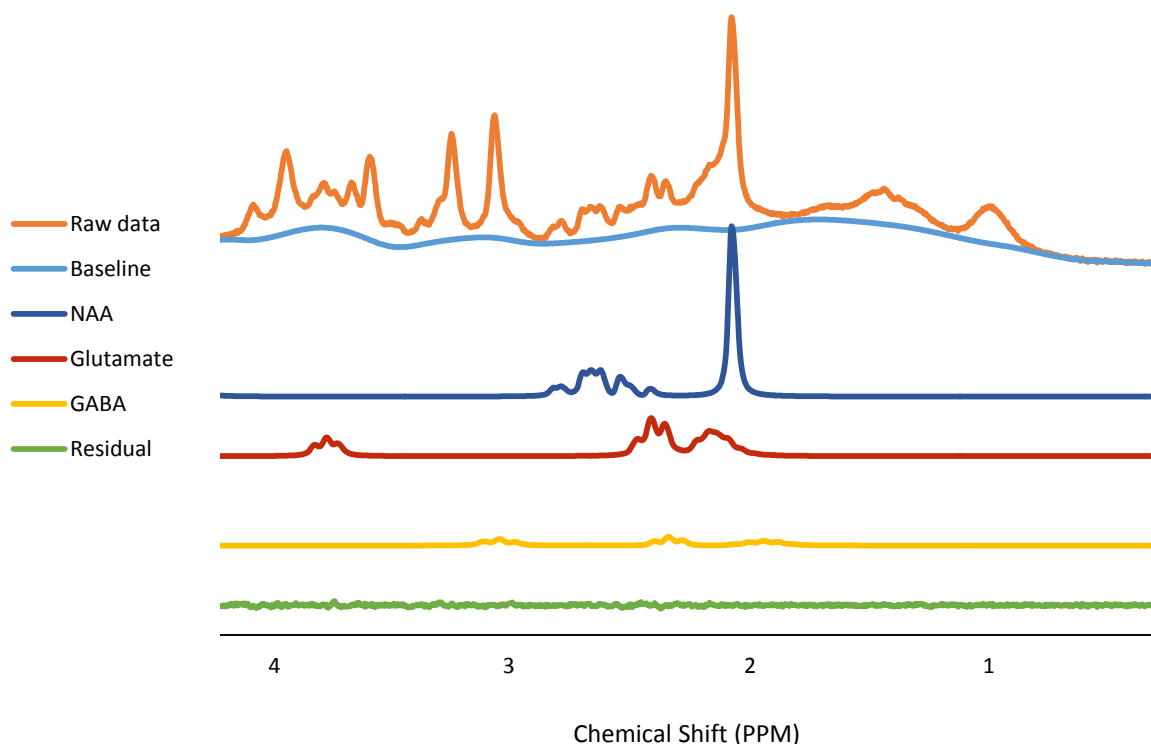


Figure 21. Example of spectrum from one participant showing raw data (orange), baseline signal (light blue) and residual signal (green). Signals for metabolites of interest are also shown including glutamate,  $\gamma$ -amino butyric acid (GABA) and N-acetylaspartate (NAA).

### 8.2.5. Spectral quantification

Spectral quantification of Glu, GABA and NAA was carried out using LCModel which provides a fully automatic analysis of in vivo spectra (Provencher, 1993). This analysis package analyses spectra as a linear combination of modelled in-vitro spectra and uses an internal basis set that consisted of 21 simulated metabolite basis spectra (Simpson et al., 2017) to fit data by phasing, referencing and quantifying metabolites. Eddy current correction was also applied by using the unsuppressed water spectrum collected for each participant.

All metabolite concentrations were evaluated as ratios to Cr (PCr + Cr). To ensure that any differences in metabolite/Cr can be interpreted as changes in the metabolite rather than Cr, it was verified that Cr concentration did not differ as a function of chronotype. To do this, Cr was measured using water as an internal reference to calculate its absolute concentration within the MRS voxel. The water signal was corrected for CSF volume and T<sub>2</sub> decay. There was no significant correlation between concentrations of Cr and rMEQ score ( $r_s(38) = -.18$ ,  $p = .26$ ). Grey matter (GM) and white matter (WM) fractions within the MRS voxel were calculated by segmenting the T<sub>1</sub>-weighted structural images using FSL FAST (Y. Y. Zhang et al., 2001) to estimate the composition of the voxel as numbers of neurones and oligodendrocytes, and therefore neurometabolites levels, differ between GM and WM. As expected, neither GM fraction ( $r_s(38) = .02$ ,  $p = .90$ ) or WM fraction ( $r_s(38) = -.27$ ,  $p = .092$ ) were correlated with chronotype.

#### 8.2.6. Statistical analyses

Statistical analyses were performed using SPSS version 24. The data were first assessed for the assumptions of normality by applying the Shapiro-Wilk test. Parametric data were assessed using Pearson's correlation coefficient and non-parametric data were analysed using Spearman's Rho correlation coefficient, where appropriate. A partial correlation was performed in order to investigate the association between chronotype and metabolite concentrations with age, gender, sleep quality (PSQI), mood (total PHQ-4 score) and time of scan as covariates. The significance level was set at  $p < .05$ .

## 8.3. Results

### 8.3.1. Participants

Participant characteristics are presented in *Table 9*. Measures of anxiety symptoms ( $r_s(40) = .087, p = .59$ ), depressive symptoms ( $r_s(40) = .123, p = .45$ ) and time of scan ( $r_s(40) = .067, p = .68$ ) were not significantly correlated with rMEQ. As with previous chapters, no systematic bias was observed between the participant's chronotype and the time of day the experiment was performed, i.e. earlier and later chronotype were equally likely to attend morning or afternoon/evening sessions. Chronotype scores were similar between male and female participants (independent samples  $t$ -test ( $t(11.57) = .020, p = .98$ )). However, similar to previous chapters, there were moderate significant correlations between rMEQ and PSQI score ( $r(40) = 0.323, p = .042$ ) and age ( $r(40) = -0.369, p = .019$ ) such that later chronotype was associated with better sleep quality and older age.

*Table 9. Descriptive statistics and basic demographics.*

Variable	M (SD)	Range (this sample)	Range (original scale)
Age	21.68 (4.08)	18-37	-
Gender	Female 30 (75%); Male 10 (25%)	-	-
Sleep quality	7.43 (3.52)	1 - 15	0 – 21
Chronotype	12.38 (3.52)	6 - 20	4 – 25
PHQ-4 (Anxiety)	1.63 (1.48)	0 - 5	0 – 6
PHQ-4 (Mood)	0.85 (1.10)	0 - 6	0 -6

*Note. Values show mean (SD). Also included are the questionnaire range (minimum-maximum) for the study sample and original scale. Sleep quality (PSQI = Pittsburgh Sleep Quality Index), chronotype (rMEQ = reduced Morningness Eveningness Questionnaire), mood and anxiety (PHQ-4 = Patient Health Questionnaire).*

### 8.3.2. Metabolite concentrations

The Shapiro-Wilk test revealed the data (levels of glutamate, GABA and NAA) did not meet the assumptions of normality ( $p < .05$ ) therefore non-parametric tests were applied. A spearman's Rho correlation demonstrated that glutamate concentration was not associated with age ( $r_s(40) = -.192, p = .24$ ), sleep quality ( $r_s(40) = .005, p = .98$ ), time of scan ( $r_s(40) = .149, p = .36$ ) or total mood and anxiety symptoms  $r_s(40) = .149, p = .36$ ). Similarly, GABA concentration was not associated with age ( $r_s(40) = -.076, p = .64$ ), sleep quality ( $r_s(40) = -.172, p = .29$ ), time of scan ( $r_s(40) = .123, p = .45$ ) or total mood and anxiety symptoms  $r_s(40) = -.100, p = .54$ ). Similarly, NAA concentration was not associated with age ( $r_s(40) = -.007, p = .97$ ), sleep quality ( $r_s(40) = -.15, p = .36$ ), time of scan ( $r_s(40) = -.05, p = .76$ ) or total mood and anxiety symptoms ( $r_s(40) = .055, p = .74$ ).

A Mann-Whitney U test also revealed no differences in glutamate, GABA and NAA levels between males and females (glutamate:  $U = 140, p = .77, 1.04$  vs.  $1.07$ ; GABA:  $U = 139, p = .75, 0.33$  vs.  $0.32$ ; NAA:  $U = 112, p = .25, 1.55$  vs.  $1.61$ ).

There was no significant correlation between chronotype and glutamate ( $r_s(33) = -.05, p = .78$ ) GABA ( $r_s(33) = .07, p = .66$ ) or NAA ( $r_s(33) = .18, p = .29$ ) concentrations, controlling for age, gender, sleep quality, mood and time of scan (see *Table 10* and *Figure 22*).

Table 10. Median metabolite concentrations across all participants.

Metabolite of Interest	Median concentration (metabolite: TCr ratio)	Partial correlation with rMEQ score (p-value)
Glutamate	1.067 (1.03 – 1.12)	p = .78
GABA	0.323 (0.29 – 0.37)	p = .66
NAA	1.586 (1.52 – 1.70)	p = .29

Note. Values show median (interquartile range) metabolite concentrations referenced to total creatine (TCr). P-values from Spearman's Rho partial correlation with rMEQ score are also displayed where age, gender, PSQI score, mood symptoms and time of scan were added as covariates. GABA = gamma-amino butyric acid, NAA = N-Acetyl aspartic acid.

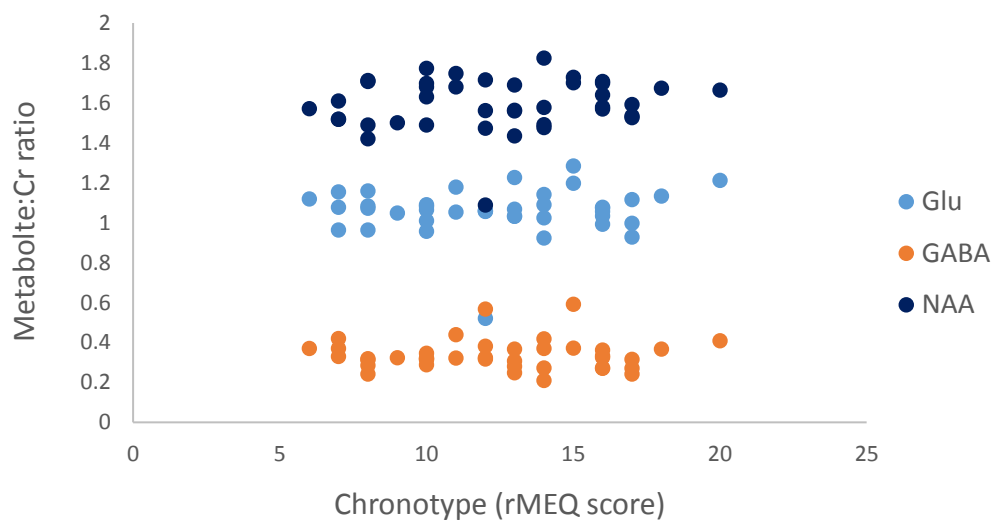


Figure 22. Scatterplot showing non-significant relationships between chronotype and glutamate (light blue circles), GABA (gamma-amino butyric acid, orange circles) and NAA (N-Acetyl aspartic acid, dark blue circles) metabolites referenced to Cr (creatine).

## 8.4. Discussion

This is the first study, to my knowledge, to explore changes in neurometabolites in the occipital cortex and relate these to chronotype. No significant correlations were observed between chronotype and GABA, glutamate or NAA levels. Previous evidence has identified late chronotype to be associated with increased prevalence of depression (Antypa et al., 2017; Antypa et al., 2016; Haraden et al., 2017; Hidalgo et al., 2009; Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013) and reduced concentrations of GABA and NAA and elevated levels of glutamate have been reported in occipital cortex of depressed patients (Bhagwagar et al., 2007; Romeo et al., 2018; Sanacora et al., 2004) and in individuals at increased risk for depression (Taylor et al., 2011). Here, no association was observed between chronotype and neurometabolite levels in the occipital cortex region of interest which suggests occipital GABA, glutamate and NAA do not play a role in risk for depression in relation to chronotype.

Previous research has largely reported reductions in occipital cortex GABA in depression (Price et al., 2009; Sanacora et al., 2004; Sanacora et al., 1999). However, other studies have reported no differences (Abdallah et al., 2014; Godlewska et al., 2015; Murrough et al., 2010) which could be explained by patient heterogeneity. For example, Sanacora and colleagues (2004) reported that the strongest reductions in GABA observed in their sample of depressed individuals was observed in a sub-set of participants with melancholic features i.e. symptoms of anhedonia or lack of mood reactivity. However, participants with atypical depression (a different subtype) did not have significantly reduced GABA levels compared to healthy controls (Sanacora et al., 2004), suggesting symptom severity or a specific symptom profile may be more related to changes in GABA than a diagnosis of depression as a whole. Moreover, Price and colleagues (2009) reported reduced levels of GABA in ACC and occipital cortex in treatment-resistant depressed participants compared to controls, and not in treatment-

naïve or less treatment-resistant participants (Price et al., 2009). Additionally, remitted patients (Shaw et al., 2013) and healthy individuals with a familial risk for depression (Taylor et al., 2011) or high neuroticism (Goto et al., 2010) do not show differences in occipital GABA levels compared to healthy controls. This further supports the evidence that reduced GABA is associated with a state marker of depression and in particular, a specific set of depressive symptoms or treatment resistance. Therefore, this may explain the lack of association between GABA and late chronotype observed in the present study.

In relation to glutamate, studies of depression have reported fewer consistent findings in the occipital cortex. Some studies report increased concentrations of glutamate or Glx (the composite measure of glutamate and glutamine) (Bhagwagar et al., 2007; Sanacora et al., 2004) whereas other studies report no differences at all (Godlewska et al., 2015; Murrough et al., 2010; Price et al., 2009). Again, this could be due to patient heterogeneity as a recent meta-analysis of <sup>1</sup>H-MRS studies of patients with depression reported that reductions in Glx in the PFC was associated with symptom severity and chronicity (Arnone, Mumuni, Jauhar, Condon, & Cavanagh, 2015). Increases in glutamate in occipital cortex have also been reported in healthy participants with familial risk for depression (Taylor et al., 2011) and increases in Glx in recovered patients (Bhagwagar et al., 2007). It has therefore been suggested that small increases in levels of glutamate in at-risk individuals are a possible trait marker for depression, which are further elevated in acute depression. However, it is noteworthy that Taylor and colleagues (2011) specifically report measures of glutamate (similar to the present study) whereas Bhagwagar and colleagues (2007) and other MRS studies of depression (Murrough et al., 2010; Price et al., 2009) only report changes in Glx (composite measure of glutamate and glutamine) which could reflect changes in glutamate or glutamine. Therefore, differences in the way that metabolites are reported could also reflect some of the discrepancies between studies. Moreover, changes in static measures of MRS glutamate or Glx are difficult to interpret because

of glutamate-glutamine cycling which occurs dynamically between neurones and astrocytes. Indeed, Abdallah and colleagues (2014) reported that depressed patients showed a 26% reduction in mitochondrial energy production using  $^{13}\text{C}$ -MRS compared to healthy controls, but no differences in glutamate or glutamine levels. The authors suggested that this could represent reduced synaptic strength between glutamatergic neurones (Abdallah et al., 2014). Therefore, another explanation for the different observations of glutamate/Glx between studies in depressed and at-risk individuals could be abnormal mitochondrial production in glutamatergic neurones. It is therefore also possible that late chronotypes have reduced synaptic strength between glutamatergic neurones without observable differences in glutamate, although this was not measured in the current study.

Alternatively, it may be that familial risk for depression, and remission from depression, is associated with altered glutamate neurotransmission in occipital cortex, but this is not a mechanism associated with risk in late chronotypes. In support of this idea, one study using gene expression analysis reported 28 probe sets corresponding to genes implicated in glutamatergic neurotransmission that were differentially expressed across the brain in subjects that had committed suicide with major depression compared to controls (Sequeira et al., 2009). In particular, the *GLUL* gene (responsible for coding an enzyme need for glutamate recycling) was found to be consistently down-regulated in depressed suicide subjects in both anterior and subcortical regions including the prefrontal cortex and amygdala, consistent with some MRS studies reporting reduced glutamate in these regions in depression (Hasler et al., 2007; Michael et al., 2003a; Auer et al., 2000; Zhang et al., 2013; Michael et al., 2003b). Differences in expression of genes relating to the GABAergic system were also reported in relation to major depression (Sequeira et al., 2009). Therefore, it is more likely that alterations in occipital glutamate (and GABA) may represent a vulnerability marker in relation to genetic risk for depression but not late chronotype.



In relation to NAA, discrepant findings have been reported in depression with either reports of reductions in frontal regions or no differences at all. Individuals at-risk of depression by virtue of high neuroticism (Ryman et al., 2011) and experience of childhood abuse (Raparia et al., 2016) have also been associated with reduced NAA levels in prefrontal regions. Similar to changes in GABA and glutamate, this has been suggested to be related to chronicity. For example, Brambilla and colleagues (2005) reported significantly reduced NAA levels in DLPFC of chronically ill depressed patients compared to patients with a less chronic form of depression and healthy controls (Brambilla et al., 2005), and NAA levels have been related to duration of illness as well (Michael et al., 2003a). Evidence of reductions in NAA in the occipital cortex are much more limited. One study of depressed patients (Sanacora et al., 2004) and one study of healthy individuals with familial risk (Taylor et al., 2011) reported no differences, similar to the present findings. One study of recovered participants showed a reduction in occipital NAA (Bhagwagar et al., 2007), however participants in this study had only been medication-free for three months and so this finding could reflect a lasting effect of antidepressant treatment; known to alter levels of NAA (Gonul et al., 2006). Therefore, the evidence suggests that reductions in NAA levels (reflecting neuronal viability) in the occipital cortex are present as a consequence of depression and is not related to chronotype.

#### 8.4.1. Limitations

One limitation has been identified that is specific to this 1H-MRS study. Measures of GABA, glutamate and NAA have been localised to the occipital cortex. This was in order to provide direct comparisons with some previous MRS studies and was used as a surrogate for whole brain resting metabolite levels whilst ensuring good spectra quality. However, the findings cannot therefore be generalised to anterior regions which are involved in emotion regulation and are more relevant to major depression. Future studies targeting anterior (e.g.

dACC and DLPFC) and limbic regions (e.g. the amygdala, where changes in functional activation were identified in chapter 5) are therefore needed to delineate changes in neurometabolites in later chronotypes, although the amygdala is a challenging brain region to study using MRS. Secondly, there is still some dispute in the MRS literature as to whether reporting metabolite values scaled to Creatine or the water peak (absolute values) is optimal. Here, metabolite values were scaled to creatine and differences in these values were not shown to be a result of underlying changes in creatine. However, some researchers argue that reporting absolute values avoids this problem altogether and so this could be taken into consideration in future studies.

## 8.5. Conclusion

In conclusion, no association was found between late chronotype and GABA, glutamate or NAA levels in occipital cortex. Although late chronotype is a recognised risk factor for depression, the current findings suggest alterations in neurometabolites do not represent a neural vulnerability marker in this population. It is more likely that changes in brain biochemistry in the occipital cortex represent the depressed state including severe symptoms and treatment resistance, or alterations in the glutamatergic and GABAergic systems are related to genetic risk for depression. However, well-powered studies probing anterior and limbic regions relevant to depression are needed in the future.

# CHAPTER NINE

## General Discussion

### 9.1. Summary of findings

As discussed in the Introduction (Chapter 1), late chronotype has been identified as a risk factor for depression (Antypa et al., 2017; Antypa et al., 2016; Haraden et al., 2017; Hidalgo et al., 2009; Merikanto et al., 2015; Merikanto et al., 2013; Vetter et al., 2018). For example, Kitamura and colleagues (2010) reported that individuals with depression were almost twice as likely to be late chronotypes than early or intermediate chronotypes after adjusting for sleep-related factors (odds ratio = 1.92) (Kitamura et al., 2010). Similarly, late chronotype has been shown to be a significant predictor of elevated depressive symptoms and a diagnosis of depression (Haraden et al., 2017; Van den Berg et al., 2018; Vetter et al., 2018). However, the underlying factors that explain why these individuals are at increased risk for depression have largely been neglected until now. The aim of the current research was therefore to extend previous knowledge of cognitive vulnerability factors to investigate the behavioural and neural mechanisms underlying this association. Overall, the current body of research has provided evidence for a number of observed behaviours and neural mechanisms attributing to impaired emotion processing and reward sensitivity in healthy individuals with a late chronotype.

In the behavioural experiments (Chapters 2 and 3), emotional processing biases were demonstrated across a range of cognitive tasks including recognition and recall of self-referent information and interpretation of facial expressions. In general, late chronotype individuals displayed a pattern of biases towards negative information, such as sad facial expressions, and

away from positive information, such as agreeable personality trait descriptors compared to early/early-intermediate chronotype individuals. The observed emotional biases in late chronotypes are similar to those reported in depressed individuals including evidence for enhanced memory for negative words, enhanced accuracy to recognise negative emotion facial expressions and a tendency to label negative information as self-referential (Matt et al., 1992; Karin Mogg & Bradley, 2005; Sloan et al., 2001). Depression is characterised by increased processing of negative stimuli and decreased processing of positive stimuli because this information is thought to be mood-congruent. Similarly, depressed mood is associated with deeply encoded negative self-schemas which results in faster processing and recall of mood-congruent information relating to the self. These biases have been shown to exist verbally (e.g. words) and socially (facial expressions) and are thought to lead to negative interpretations of the environment and social interactions (Beck & Bredemeier, 2016). Therefore, the current findings of negative emotional biases in late chronotypes suggests that these individuals have negative self-schemas and negative interpretations of external stimuli similar to depressed individuals.

In comparison, selective memory and interpretation of negative information was not accompanied by attentional biases to negative stimuli. There is some evidence that attentional biases in depression represent impaired initial processing of negative information which leads to further negative processing of the stimulus (Hill & Dutton, 1989). However, findings of attentional biases in depression have been mixed and are more reliably revealed when stimuli are presented for longer periods of time suggesting a difficulty in disengaging from negative emotional stimuli rather than attending to stimuli (Mogg et al., 1995b). This suggests that risk for depression is either not categorised by attentional biases in late chronotype individuals or that the experimental design (dot-probe task) did not capture disengagement from emotional information that may be more relevant to depression.

Interestingly, late chronotypes also failed to recall as many personality descriptors overall (irrespective of emotional valence) compared to early-intermediate chronotypes. This finding was unexpected but could represent an indirect measure of global memory impairments, similar to that seen in depressed individuals which are thought to be partly related to impairments in motivation (Austin et al., 2001; Kindermann & Brown, 1997). Therefore, there is some evidence that memory impairments and amotivation may be involved in risk for depression in late chronotype individuals and future studies using direct measures of these constructs are needed.

Finally, late chronotypes displayed reduced reward sensitivity during a risk-taking task compared to early-intermediate chronotype individuals indicating a conservative response bias. This finding was present despite normal overall risk-taking behaviour suggesting a specific abnormality in processing reward. Similar findings of impaired reward-seeking and/or enhanced sensitivity to punishment have been reported in depression and have been related to emotional blunting and symptoms of anhedonia often reported by depressed individuals (Hevey et al., 2017). Therefore, the current findings of impaired sensitivity to reward observed in late chronotypes suggests emotional blunting may be an important marker underlying risk for depression. It also may explain why late chronotype is associated with a number of risky behaviours e.g. smoking, alcohol use, sexual behaviour and gambling (Adan, 1994; Taillard, Philip, & Bioulac, 1999; Wang & Chartrand, 2015), i.e. individuals feel they need to take bigger/more risks for the same amount of reward. The current findings also highlight disparities between self-report measures of risk-taking (which often highlight increased risk-taking in late chronotypes) and behavioural measures of risk-taking reported here (BART task), suggesting more sensitive measures of reward and punishment sensitivity may be more relevant in explaining risk for depression in this population.

Taken together, behavioural evidence from Chapters 2 and 3 indicate emotional biases towards negative information and away from positive information in late chronotype individuals, similar to depressed patients. Negative attentional biases may not be present in this at-risk group but un-expectantly, global memory impairments were revealed that may be relevant to vulnerability for depression. Finally, late chronotype was characterised by impairments in reward sensitivity but not overall risk-taking. Complementary to these findings, one study modelling the relationship between chronotype and depression using path analyses suggested a two-step model: later chronotypes reported less reward responsiveness, which was associated with lower levels of positive affect and higher levels of negative affect, which in turn was associated with increased depressive symptomatology (Hasler, Allen, Sbarra, Bootzin, & Bernert, 2010). Therefore, the current integrated behavioural findings may represent vulnerability markers for depression in relation to late chronotype.

In addition to the behavioural evidence, the neural substrates of emotional processing biases were demonstrated using fMRI (Chapter 5). Later chronotype was associated with enhanced amygdala reactivity to fearful vs. happy facial expressions and reduced functional connectivity between the amygdala and dorsal Anterior Cingulate Cortex. This finding indicates an enhanced neural response to negative emotional stimuli coupled with reduced emotion regulation suggesting a neural mechanism underlying biased emotional processing of facial expressions. This finding complements the behavioural findings in a number of ways. Firstly, the task used in the fMRI experiment was adapted and simplified from the task used in the behavioural studies (Chapters 2 and 3) as participants were asked to discriminate the gender of the face rather than explicitly categorising the emotional expression. This suggests that negative biases to facial expressions exist when facial expressions are processed both implicitly and explicitly. Secondly, both experiments analysed responses to prototypical faces (i.e. 100% emotion) but the behavioural paradigm further revealed that differences exist between

chronotype groups with different emotional intensity levels, particularly when facial expressions were ambiguous. Third, happy and fearful expressions were selected for the fMRI task (rather than happy and sad) which was based on previous evidence for enhanced response to threat in depression (Sheline et al., 2001). This suggests processing biases away from both negative emotions (fearful and sad) and towards positive emotions (happy) in late chronotypes. Finally, the fMRI findings indicate that impaired emotion regulation by the dACC may be contributing towards the observed emotion processing biases in late chronotypes suggesting further impairments in the mood regulation circuit. Therefore, integrating findings across fMRI and behavioural experiments contributes towards our understanding of how negative biases towards facial expressions are associated with negative interpretations of social interactions.

In relation to resting-state connectivity (Chapter 7), later chronotype was associated with decreased connectivity in the Posterior Cingulate Cortex and Precuneus within the Default Mode Network. These brain regions have been associated with self-referential processing and attentional processing, and depression has been associated with impaired DMN connectivity and functional activation of these regions (Brakowski et al., 2017). As noted above, the behavioural findings indicate mood-congruent recognition and recall biases for self-referent information in late chronotypes suggesting these individuals have self-critical thoughts. Therefore altered DMN resting-state connectivity may represent a neural mechanism underlying self-critical cognitive biases in late chronotypes. Alternatively, altered connectivity within the PCC/precuneus could be related to a reduced ability to allocate attentional resources between internal and external sources. Attentional biases towards emotional information were not observed in the behavioural experiment in late chronotypes although as discussed above, the current task may not have been optimised to reveal disengagement from stimuli specifically. Altered connectivity within this brain network at rest may therefore represent a neural

mechanism underlying self-critical thoughts or altered attentional processing at rest in late chronotypes, similar to altered resting-state connectivity observed in depressed individuals.

Global structural differences in brain regions involved in emotional processing were not observed in relation to later chronotype although a non-significant trend towards inward deformation over the subiculum of the hippocampus was observed using morphology analysis (Chapter 6). Reduced hippocampal volume and altered hippocampal morphology have been largely reported in depressed patients and neuronal loss in this structure is thought to contribute towards impaired emotion processing (Videbech & Ravnkilde, 2004). The current finding therefore suggests that risk for depression is more closely related to functional neural abnormalities in emotion processing in late chronotypes rather than structural differences which is consistent with the neurotoxicity hypothesis. Similarly, risk for depression in this population was not related to global neurometabolite changes measured in occipital cortex (Chapter 8). In particular, decreased levels of occipital GABA have been reported in depression which is thought to represent a global reduction in GABA synthesis and decreased function of the inhibitory neurotransmitter. The current finding suggests that impaired neural mechanisms responsible for biased emotion processing in late chronotypes are not related to global differences in GABA, Glu and NAA. However, neurotransmitter changes in brain regions more closely associated with emotion processing (e.g. ACC, DLPFC) have yet to be investigated in this population. Together these findings suggest structural abnormalities (such as reduced hippocampal volume) and neurochemical differences (such as decreased occipital GABA concentrations) are associated with the depressed state rather than risk for depression in relation to chronotype, and may only develop should depression be triggered.

The current findings make a significant contribution to the existing literature. The behavioural data partially replicate evidence for increased processing of negative information and decreased processing of positive information and extend this observation across a range of



emotion-processing tasks whilst providing new evidence for reduced reward sensitivity in late chronotype individuals. The neural substrates underlying negative processing of facial expressions were revealed using fMRI and a pattern of altered connectivity at rest was revealed using resting-state fMRI in relation to chronotype. Structural and neurometabolite differences were not found to be related to late chronotype in this sample. These neural findings, to the best of my knowledge, are some of the first (if not the first) to investigate neural mechanisms underlying risk for depression in relation to late chronotype and therefore complement and extend our current knowledge. Importantly, participants were healthy individuals that had never been depressed, had never been diagnosed with a sleep disorder and did not have a family history of depression, meaning the current findings represent trait markers for depression.

As discussed in Chapter 1, a converging corpus of research has provided evidence that late chronotype is a risk factor for major depression, specifically severe symptoms of low mood (Levandovski et al., 2011; Merikanto et al., 2015; Merikanto et al., 2013). Evidence suggests that the effect size for this relationship is small ( $Z = 0.2$ ) when confounding factors such as age, gender, sleep quality and health factors are taken into consideration (Au and Reece., 2017) but late chronotype is thought to be largely independent of other established risk factors such as neuroticism, familial risk, sleep disturbances and female gender. Although the relationship is weak, the current integrated findings are therefore thought to represent behavioural and neural vulnerability markers for depression/low mood specifically related to late chronotype. Importantly, these differences may help to explain why these individuals are at increased risk of developing the disorder. However, it is important to note that the previous literature is mainly cross-sectional and the present findings do not speak to the causal nature of late chronotype as a risk factor for the development of depression. Similarly, it is unclear whether late chronotype presents as a pure risk factor or whether this trait contributes to increased susceptibility to stress which in combination increases the risk for depression. Therefore, future longitudinal studies

following up participants over a number of years and recording chronotype and diagnoses of depression are needed. Nonetheless, the current findings represent some of the first indications that altered emotion processing and reward seeking may underlie the relationship between late chronotype and low mood.

The primary aim of the current research was identify mechanisms association with risk for depression in relation to late chronotype. However, the findings also suggest that positive biases may exist in relation to early chronotype. For example, early-intermediate chronotypes recalled significantly more positive than negative personality trait words which was not observed in late chronotypes. Similarly, earlier chronotype was specifically associated with enhanced amygdala reactivity to happy facial expressions and reduced reactivity to fearful facial expressions. Indeed, a recent large-scale study indicated that healthy individuals with an early chronotype were significantly less likely to develop depression over 4 years compared to late and intermediate chronotypes (Vetter et al., 2018). Therefore the current findings could be interpreted as representing protective factors for the development of depression associated with early chronotype (or early-intermediate chronotype). Future studies further characterising neurocognitive mechanisms associated with low risk (or resilience) for depression are required.

## 9.2. Experimental design

The current research has provided a large amount of data from six cross-sectional studies. The behavioural experiments were designed to employ a wide range of tasks to assess emotion processing and reward-related behaviours hypothesised to be associated with risk for depression. The fMRI experiment was used to complement the facial expression recognition task and investigate the neural mechanisms underlying emotion processing biases. In addition, the structural MRI, resting-state fMRI and MRS chapters were designed to explore a variety of

mechanisms associated with risk for depression using a multi-modal approach and again were employed to complement the behavioural findings. In contrast, the majority of previous research has been restricted to a specific domain (e.g. memory, attention) or method (e.g. fMRI). The current design therefore allowed neuropsychological mechanisms underlying vulnerability for depression in late chronotypes to be explored and integrated within a single model.

As noted above, participants were excluded if they reported a current or previous diagnosis of depression. According to this criterion, only healthy participants were recruited which allowed risk for depression to be specifically investigated. This also allowed individuals that had previously experienced depression (but could presently be healthy) to be excluded as these individuals are at increased risk for depression relapse (remitted depression) and may have so-called ‘scar effects’ of depression (Visted, Vollestad, Nielsen, & Schanche, 2018). Individuals that reported a family history of depression or a diagnosed sleep disorder were also excluded as these represent independent risk factors for depression (Beardslee, Versage, & Gladstone, 1998; Morphy, Dunn, Lewis, Boardman, & Croft, 2007). In addition to this, measures of subclinical depressive symptoms, anxious symptoms and sleep quality were obtained from all participants in order to statistically control for and exclude these effects on the current findings. A measure of neuroticism personality trait was collected in some experiments (Chapter 2) as high neuroticism has also been shown to be a risk factor for depression (Kendler, Gatz, et al., 2006). Not all experiments were able to exclude the effect of neuroticism (measure not collected in neuroimaging chapters) although this trait was not found to influence the behavioural findings and late chronotype has not been consistently related to high neuroticism (Hogben et al., 2007) suggesting the current findings were not driven by differences in neuroticism. Similarly, measures of smoking and alcohol use were only collected for the behavioural study (Chapter 2). Some previous evidence has shown that smoking,

increased alcohol consumption and drug use are independent risk factors for depression (Brook et al., 2002) and so could be confounding variables in the current studies. In Chapter 2, there was no significant difference between self-reported number of cigarettes smoked and number of alcoholic units consumed per week between late and early-intermediate chronotype groups and overall the mean values were very low (mean number of cigarettes = 0.75, mean units of alcohol = 2.54). This suggests that the current findings were not driven by smoking and alcohol use however measures of drug use were not collected and so future studies should obtain these measures in order to fully exclude their effects on the current findings. Age, gender and sleep quality were associated with chronotype in some experiments (see section 9.3. for discussion) but were controlled for in each experimental design. Therefore, the current findings are largely thought to represent altered emotion processing, reward and neural mechanisms associated with late chronotype and its specific contribution to risk for depression.

The current design measured and took into consideration experimental timing which has often been neglected in previous studies. Performance on a number of cognitive tasks is known to show variation across the day including; attentional capacity, executive function and memory (Schmidt et al., 2012). Additionally, there is evidence that both the BOLD response during cognitively-demanding tasks (Reske et al., 2015; Rosenberg et al., 2015; Schmidt et al., 2015) and resting-state connectivity (Hodkinson et al., 2014) show diurnal variation. Similarly, there is evidence from animal models that neurotransmitters (e.g. dopamine, GABA and glutamate) are under circadian control too (Castaneda, de Prado, Prieto, & Mora, 2004). Previous studies have shown that some tasks may be sensitive to chronotype x time effects (synchrony effects) (Song et al., 2017) i.e. performance or functional activation is altered depending on the individual's optimum time-of-day (late chronotypes in evening, early chronotypes in morning). Therefore, controlling for the time of experiment in analyses is important to assess only chronotype-specific effects on emotion processing, reward and brain function. Indeed, when

adding the time of experiment as a covariate in statistical analyses, many of the main effects of attention, recognition and recall on accuracy and reaction time were influenced by this factor. Therefore, the current findings are largely independent of synchrony effects.

### 9.2.1. Weaknesses

Weaknesses relating to experimental design and methodology specific to each experiment are reported in the associated chapter (Chapters 2, 3, 5, 6, 7, 8). However a number of limitations common to all experiments should be taken into consideration when interpreting findings. Firstly, chronotype was determined using a single brief self-report metric (the rMEQ or MEQ (Chapter 2)). This metric has been widely used in previous chronobiology research and ratings obtained using this tool correlate well with biological measurements of chronotype (Baehr et al., 2000; Burgess & Fogg, 2008; Di Milia et al., 2013; Vincenzo et al., 2006). However, it is generally considered that using objective measures of chronotype is a more valid approach to measuring this trait and so future studies may benefit from using additional measures; for example, core body temperature, estimates of melatonin and cortisol levels, polysomnography, sleep diaries and actigraphy. Secondly, current, previous and family history of depression and sleep disorders was determined using self-report which relies on the participant's honesty and knowledge of mental health diagnoses. Therefore, there is a possibility that participants that have not met the inclusion criteria were included in analyses. Future studies may benefit from reference to medical history or structured clinical interview to assess exclusion criteria. Thirdly, although the time of experiment was statistically controlled for in analyses, due to practical considerations it was not ensured that participants were in a similar circadian phase by fixing experiment times relative to individual wake times. As noted above, a range of cognitive functions have been shown to vary across the day (Blautzik et al., 2013; Reske et al., 2015; Rosenberg et al., 2015; Schmidt et al., 2015; Song et al., 2017) and

this has been shown to be related to an individual's chronotype (Hahn et al., 2012). Therefore, future studies would benefit from explicitly controlling experiment timing in order to rule out any possible (a)synchrony effects or to explore chronotype x time interactions further. In addition, females were included in the present studies and were not excluded for use of oral contraception. There is some evidence that ovarian hormonal fluctuations and oral contraception alter the BOLD response to facial expressions (Marečková et al., 2014) and verbal/mental rotation tasks (Dietrich et al., 2001) in brain regions responsible for emotion processing and cognition (Toffoletto, Lanzenberger, Gingnell, Sundström-Poromaa, & Comasco, 2014). Future studies controlling for the stage of menstrual cycle, excluding women taking hormonal contraceptives or studying males and females separately are needed.

Finally the current research included only a cross-sectional investigation of the behavioural and neural mechanisms underlying risk for depression in relation to late chronotype. Although the findings provide a wealth of new information by comparing mechanisms between chronotype groups at a single time point, they do not speak to the predictive power of these observations. Indeed, most of the current research investigating the association between chronotype and major depression is cross-sectional and only a few studies to date have followed individuals over a time period using a longitudinal design (Haraden et al., 2017; Van den Berg et al., 2018; Vetter et al., 2018). Therefore, the current research cannot provide an estimate of how likely the observed emotion processing biases can be used to predict subsequent depression.

### 9.3. Study samples

The current study recruited healthy participants and measured their chronotype to define risk for depression. Chronotype is a relatively stable trait that has been recognised as a risk

factor for depression (Merikanto et al., 2015; Roenneberg et al., 2007). Three separate samples were used across studies: behavioural data (Chapter 2), data collected online (Chapter 3) and neuroimaging data (Chapters 5 – 8). This was because larger sample sizes were needed to assess cognitive biases in the behavioural experiments and collecting data online presented a good opportunity to acquire a larger sample ( $n = 226$ ). The majority of participants recruited to the first behavioural experiment and neuroimaging experiments were undergraduate students from the University of Roehampton or Royal Holloway University. Conducting the second behavioural experiment online therefore allowed negative cognitive biases to be investigated in a more diverse sample. Observations surrounding participant demographics across all three samples (summarised in *Table 11*) will be discussed here.

Across all three samples, a range of chronotypes were recruited which approximately followed a normal distribution consistent with previous observations (Fischer, Lombardi, Marucci-Wellman, & Roenneberg, 2017). During recruitment, particular effort was made to attract individuals with an extreme chronotype to participate; particularly late chronotypes. Extreme early chronotypes were difficult to recruit from the student population available but the full range of chronotypes was largely represented across samples. Moreover, during the behavioural experiments, the chronotype groups were determined using the recommended cut-off points from the original MEQ and rMEQ questionnaires (Adan & Almirall, 1991a; Horne & Ostberg, 1976b) rather than a median split. Therefore, the current results are believed to represent risk for depression in a well-defined ‘late chronotype’ group.

*Table 11. Comparisons of demographic data compiled from the three separate samples.*

	<b>Behavioural data (Chapter 2) N = 93</b>	<b>Online data (Chapter 3) N = 226</b>	<b>Neuroimaging data (Chapters 5 – 8) N = 50</b>
<b>Chronotype</b>	43.5 16 – 80 MEQ scale: 16 – 86	13.9 6 – 24 rMEQ scale: 4 – 25	12.6 6 – 20 rMEQ scale: 4 – 25
<b>Depressive symptoms</b>	7.5 0 – 35 BDI scale: 0 - 63	1.25 0 – 5 PHQ-4 subscale: 0 - 6	0.9 0 – 5 PHQ-4 subscale: 0 - 6
<b>Anxious symptoms</b>	37.4 20 – 77 STAI scale: 20 - 80	0.87 0- 5 PHQ-4 subscale: 0 - 6	1.6 0 – 6 PHQ-4 subscale: 0 - 6
<b>Sleep quality</b>	7.7 1 – 15 PSQI scale: 0 - 21	5.3 0 – 15 PSQI scale: 0 - 21	7.3 1 – 15 PSQI scale: 0 - 21
<b>Neuroticism</b>	10.3 0 – 24 EPQ scale: 0 - 24	-	-
<b>Age</b>	20.6 18 - 50	29.4 18 - 72	21.2 18 - 37
<b>Gender F/M</b>	76/17	150/76	38/12

*Note. Apart from gender, values indicate the mean and range across the whole sample and where necessary, the questionnaire range from the original scale is presented below. Also shown is the proportion of males and females in each sample. For ease of reference, MEQ = Morningness-Eveningness Questionnaire, rMEQ = reduced MEQ, BDI = Beck's Depression Inventory, PHQ-4 = Patient Health Questionnaire (4 – item), STAI = Spielberg's State-Trait Anxiety Inventory, PSQI = Pittsburgh's Sleep Quality Index and EPQ = Eysenck's Personality Questionnaire.*



Late chronotype was not associated with higher depressive symptoms across all three samples. Previous research has reported that late chronotype is associated with higher odds for diagnosed depression as well as depressive symptoms in healthy individuals (Merikanto et al., 2015) although one study reported that risk of reporting moderate-severe depressive symptoms was reduced by 3.3.% for each year younger the individuals were (Hidalgo et al., 2009). Therefore, the current sample of young adults may have masked this association. Moreover, individuals that reported a current or previous diagnosis of depression (and therefore higher depressive symptoms) were excluded. Indeed, the mean scores of depressive symptoms were low and below the suggested cut-off points for positive screening (although the questionnaires used are not diagnostic tools) (see *Table 11*). Therefore the current findings specifically underlie risk for depression in relation to late chronotype rather than increases in subclinical symptoms. Similarly, there were no associations between late chronotype and anxious symptoms (state and trait anxiety) across samples and mean anxiety levels were low (*Table 11*). Although clinical anxiety is often reported as a comorbidity in depression, previous research has suggested that late chronotype is a specific risk factor for major depression (Antypa et al., 2016) and therefore the current findings are thought to reflect impaired cognitive and neural mechanisms associated with risk for depression in this population.

In relation to sleep quality, there was a differential association with late chronotype across samples. Late chronotype was not associated with sleep quality in the first behavioural experiment, was weakly associated with poor sleep quality in the online experiment and was moderately associated with better sleep quality in the neuroimaging experiments. Previous evidence has shown that late chronotypes are more likely to report poor sleep quality and daytime tiredness (Taillard, Philip, Coste, Sagaspe, & Bioulac, 2003) and there is some evidence that the relationship between late chronotype and depression is partly mediated by poor sleep quality (Horne et al., 2018). Indeed, poor sleep quality and sleep disturbances are

commonly associated with current depression and have been shown to be an independent predictor of depression onset (Paunio et al., 2015). Differences across samples in the present investigation are unclear but the mean PSQI scores across the samples are relatively high (*Table 11*), reflecting poor sleep quality overall despite exclusion of participants with a diagnosed sleep disorder. This observation is comparable with previous studies in similar populations (Becker et al., 2018) and most likely reflects the student population studied. Sleep quality was added as a covariate in all experiments and so weak associations between poor sleep quality and chronotype are unlikely to be driving the current findings.

The age range of samples (except the online study) was limited as the majority of participants were undergraduate students. In the neuroimaging experiments, late chronotype was associated with older age whereas previous evidence has shown the opposite association (Roenneberg et al., 2007). However, children and older adults have been shown to display earlier chronotypes whilst peak ‘lateness’ is at 19 – 20 years (Fischer et al., 2017; Randler, Freyth-Weber, Rahafar, Florez Jurado, & Kriegs, 2016) which is roughly the mean age of this sample. Indeed, later chronotype was associated with younger age in the online experiment where participants with a wider age range were included. Previous evidence has suggested that late chronotype is a risk factor for depression independent of age and so the current findings suggest that impaired cognitive and neural mechanisms represent vulnerability to depression in this population across all ages. Due to ethical reasons, the minimum age of participation in the current investigations was 18 and Sorenson and colleagues (1991) estimated that 75% of individuals that experience depression will report onset after this age (Sorenson, Rutter, & Aneshensel, 1991). This suggests the present investigations capture individuals that will later develop depression. However, late chronotype has also been shown to predict a diagnosis of depression after 1 year in adolescents (Haraden et al., 2017) and so future studies replicating

the mechanisms underlying vulnerability to depression, and the predictive power of these abnormalities, in younger samples are needed.

Finally, the present studies did not explore any interactions between sex and late chronotype in relation to the mechanisms underlying risk for depression. This was because the sex ratio was not balanced across samples as many more participants were female than male (see *Table 11*). There is evidence that depression incidence is 1.7-fold greater in females and that female sex is a risk factor for depression (Ford & Erlinger, 2004). Indeed, predictive models for the onset of depression in other at-risk populations such as high neuroticism are constructed separately for men and women (Kendler, Gardner, & Prescott, 2002, 2006). However, to date, the chronotype literature has not indicated increased risk for depression in females with a late chronotype (Antypa et al., 2017) and in general, later chronotype is associated with male sex. Therefore, the current findings are thought to represent vulnerability markers for depression independent of gender although future studies may wish to directly explore this further.

#### 9.4. Theory underlying vulnerability for depression

The causes of elevated incidence of depression in healthy, late chronotype individuals as compared to early and neither chronotypes are still largely unanswered and are likely multifactorial. The aim of the present research was to investigate negative cognitive biases and impaired neural mechanisms in relation to chronotype and has highlighted the importance of these factors in understanding risk for depression in this population. However, the question remains: *why might late chronotypes display negative cognitive biases and impaired neural mechanisms?* This section will therefore discuss developing theories that attempt to explain the complex association between late chronotype and depression.

One possible mechanism underlying the relationship between late chronotype and depression is that clock genes have overlapping functions in regulating neurotransmission and circadian rhythms. A number of neurotransmitter and neurotrophin systems implicated in the pathophysiology of depression show circadian rhythmicity including dopamine, glutamate and GABA (Castaneda et al., 2004). Similarly, diurnal variations in connectivity and regional cerebral blood flow (rCBF) have been reported in resting-state networks including the default mode network (DMN) which has been implicated in depression (Hodkinson et al., 2014). In particular, clock genes are expressed in the mood regulation circuit including in the ventral tegmental area, prefrontal cortex and amygdala, suggesting that circadian rhythms and mood regulation are interconnected. These findings support the current data: increased amygdala reactivity and reduced amygdala-dACC connectivity (Chapter 5) and altered resting-state connectivity within the DMN (Chapter 6) in relation to late chronotype, and the associated behavioural changes (Chapters 2 and 3). In addition to this, a number of mutations in clock genes have been associated with current depression. For example in one study, Soria and colleagues (2010) reported that single nucleotide polymorphisms (SNPs) in *CRY1* and *NPAS2* circadian genes were associated with unipolar depression and SNPs in *CLOCK* and *VIP* circadian genes were associated with bipolar disorder (Soria et al., 2010). Moreover, chronotype and depression share a significant proportion of genetic variation: one study estimated that 59.1% of the phenotypic correlation between chronotype and depression was accounted for by genetic covariance (Toomey et al., 2015). Therefore, risk for depression in relation to late chronotype may, in part, be explained by genetic covariance including clock genes that regulate neurotransmitter systems and circadian rhythms.

However, genetic covariance is likely to account for only a proportion of the relationship between late chronotype and depression. Another theory proposed by Merikanto and colleagues (2015) suggests that late chronotypes may be at increased risk of circadian misalignment of

biological rhythms due to a clash between their internal circadian rhythm and external demands (e.g. the working day, with few exceptions, begins between 7am and 9am). This concept has been termed ‘social jet-lag’ and may explain the increased prevalence of a number of health-related issues associated with late chronotype including depression. Evidence for this was supported by Roenneberg and colleagues (2007) that reported sleep duration was substantially shorter (1 – 4 hours) on working days and prolonged on work-free days in late chronotypes compared to early chronotypes that did not report this difference (Roenneberg et al., 2007). In the same study, late and early chronotypes were equally likely to report being long-sleepers (> 9 hours) and short sleepers (< 6 hours) suggesting that late chronotypes experience more social jet-lag independent of overall sleep duration. Moreover, in a rural population (n = 4051), late chronotype was positively correlated with degree of social jetlag (calculated from the difference between mid-sleep on free days and mid-sleep on work days) and depressive symptoms in healthy and clinically depressed individuals (Levandovski et al., 2011). Social jet-lag is thought to be a chronic stressor that has a negative impact on a number of cognitive abilities including memory, attention and decision-making. For example, degree of social jet lag was significantly associated with a poorer weekly average grade in undergraduates during term times but this association disappeared during the exam period when there were no longer any timetabled lectures (Haraszti, Ella, Gyongyosi, Roenneberg, & Kaldi, 2014). This suggests that social jet lag may act as a stressor causing negative cognitive biases to develop in late chronotype individuals.

In animal models, hamsters subjected to a paradigm mimicking jet-lag displayed impaired learning and memory and reductions in hippocampal neurogenesis (Gibson, Wang, Tjho, Khattar, & Kriegsfeld, 2010) suggesting circadian disruption may also be related to structural differences in the brain. In humans, flight attendants with short recovery periods (< 5 days) between international flights (indicating chronic jet lag) displayed reduced temporal

lobe volume, spatial learning and memory deficits and elevations in cortisol compared to flight attendants with long recovery periods (Cho, 2001). Although the relationship between social jet-lag and structural differences has not been measured directly in humans, later chronotype has been associated with greater regional grey matter density (rGMD) in precuneus and posterior parietal cortex and lower rGMD in orbitofrontal cortex (Takeuchi et al., 2015) and lower fractional anisotropy (FA) in the white matter underlying left ACC, corpus callosum and left frontal lobe using DTI (Rosenberg et al., 2014). In the present study (Chapter 7), later chronotype was not associated with GMD or hippocampal volume changes although a significant trend towards changes in hippocampal morphology was observed. Together, these findings suggest that a possible theory underlying the relationship between late chronotype and depression is that these individuals experience a higher degree of social jet-lag than early chronotypes, which acts as a stressor and causes negative cognitive biases and impaired neural mechanisms to develop.

However, some studies have also shown no association between degree of social jet-lag and depressive symptoms in relation to chronotype. For example, later chronotype was not related to increased experience of social jet lag in healthy adolescents (de Souza & Hidalgo, 2014) undergraduate students (Sheaves et al., 2016) or in a sample of depressed patients (Knapen et al., 2018). The discrepancies between findings may be because social jet lag is a difficult construct to measure (relies on participants to wake-up naturally on free days) or that its effect on depressive symptoms develops over time. Alternatively, more sensitive measures of light exposure and sleep quality (which are probably related to social jet-lag) may be more relevant in explaining increased risk for depression in relation to late chronotype. For example, another possible theory is that late chronotypes are exposed to (or are more sensitive to) more artificial blue light in the evenings compared to early chronotypes which may explain their vulnerability to depression. Indeed, Seasonal Affective Disorder (SAD) is a specific subtype of

depression that is characterised by depressed mood during winter months and is thought to be caused by lower levels of natural daylight and a shift in melatonin rhythm (Rosen et al., 1990). Artificial night time lighting has also been associated with non-seasonal depression although epidemiological studies investigating this relationship are limited. For example, shift workers are more likely to suffer from depressive episodes, and increasing exposure to shift work (more than 20 years) is associated with elevated risk for major depression (Scott, Monk, & Brink, 1997). There is also a correlation between the incidence of major depression and the adoption of electric lights whilst populations that have rejected artificial light (e.g. Amish people) have been shown to have low incidence of depression (Egeland & Hostetter, 1983). Evening light exposure is thought to disrupt mood via a number of mechanisms (Bedrosian & Nelson, 2017). For example, exposure to light at night strongly suppresses melatonin, which rises at night to promote sleep onset, and causes sleep disruption. Although only one contributing factor, sleep disturbances have been implicated in depressed mood via impaired mood regulation (Palmer & Alfano, 2017). Indeed, late chronotype has often been associated with sleep disturbances, insufficient sleep, nightmares and poor sleep quality (Yun, Ahn, Jeong, Joo, & Choi, 2015) and the relationship between late chronotype and depressive symptoms has been shown to be partly mediated by poor sleep quality in healthy individuals (Horne et al., 2018). Secondly, exposure to night-time light alters the expression of clock genes. As noted above, a number of polymorphisms in clock genes have been associated with depression independent of light exposure and have a number of downstream effects (Soria et al., 2010). Therefore, altered light exposure may predispose individuals to depression (and other mood disorders) by interacting with circadian genes.

Altered clock gene expression caused by night-time lighting is also thought to impair modulation of neurotransmitter systems which display circadian rhythms in concentration and release (Wirz-Justice, 1987). A large body of research has indicated a number of

neurotransmitter and neurotrophin systems in the neuropsychology and treatment of depression including diminished serotonin activity and reduced BDNF levels (Martinowich & Lu, 2008). The animal literature has shown the impact of altered lighting on these systems. For example, Fonken and Nelson (2013) reported reduced Brain Derived Neurotrophic Factor (BDNF) mRNA expression in hippocampus and displays of depressive symptoms in mice that were exposed to 4 weeks of dim light at night compared to mice exposed to a normal light-dark cycle (Fonken & Nelson, 2013). Similarly, Matsumura and colleagues (2015) reported that rats exposed to an artificial light cycle (6 hour light, 6 hour dark) had reduced levels of serotonin and increased levels of norepinephrine in a number of brain areas compared to rats exposed to a normal light-dark cycle (Matsumura, Nakagawa, Suzuki, Ninomiya, & Ishiwata, 2015). As noted above, Castaneda and colleagues (2004) also reported that dopamine, glutamate and GABA showed circadian rhythms in the nucleus accumbens and striatum of rats (Castaneda et al., 2004). Therefore, exposure to bright light at night may alter neurotransmitter systems by affecting expression of circadian genes, which may contribute to the underlying vulnerability to depression in late chronotype individuals. For example, a preliminary study reported that late chronotype was significantly associated with reduced overall levels of serum BDNF (measured at 2pm) in a sample of healthy adult males but no association was found in females (Eckert et al., 2018). Levels of small neurotransmitters such as serotonin and dopamine are difficult to measure in humans and have not yet been investigated in relation to chronotype. Moreover, Castaneda and colleagues also reported that only glutamate and GABA modulation in striatum were influenced by light (Castaneda et al., 2004) suggesting that only certain neurotransmitters in particular brain regions are sensitive to light. This could explain part of the reason why here, levels of occipital GABA and glutamate were not associated with chronotype (as reported in Chapter 8). Together, increased exposure or sensitivity to artificial light at night in relation to late chronotype could explain their increased vulnerability to depression through a combination



of mechanisms including sleep disturbances, altered clock gene expression, changes in neurogenesis and altered neurotransmitter modulation.

Taken together, the underlying mechanisms that explain *why* late chronotype is associated with negative cognitive biases and impaired neural mechanisms, and increased vulnerability to depression is still unknown. There is undoubtedly a very complex association and a number of intertwined factors are involved. Current findings suggest genetic covariance, social jet lag (or circadian misalignment) and increased artificial light exposure/sensitivity in late chronotypes may contribute towards risk for depression via downstream mechanisms affecting mood regulation circuitry. However, the causal nature of these factors is still unknown as it is likely that late chronotype influences light exposure, degree of social jet-lag and sleep disturbances too. The current study did not measure light exposure, genetic make-up or social jet-lag because the aim was to investigate the specific effect of late chronotype on emotion processing and reward. Therefore, the specific contributions of these factors on the current findings are speculative but it is likely that all three theories (and possibly others) are involved.

## 9.5. Implications of research

Major depression is a debilitating disorder that affects roughly 322 million people worldwide (WHO, 2017). A key aim of neuropsychological research is to identify at-risk populations and mechanisms underlying risk for depression in order to develop interventions to prevent depression onset. This is of great importance to reduce the health, social and economic burden of the disorder. In addition, late chronotype is recognised as a risk factor for depression and a recent study of the UK Biobank cohort indicated a significantly increased risk of mortality overall in this group (Knutson & von Schantz, 2018). Therefore, identifying

strategies to promote well-being in late chronotypes is also vital. Major depression is a multifaceted and complex disorder and there are likely to be a large number of mechanisms involved in its aetiology. Although the current research has highlighted a number of cognitive and neural mechanisms relevant to risk for depression in relation to chronotype, the present findings likely represent part of the mechanisms involved. Nonetheless, the current findings are believed to have important clinical and theoretical implications for the prevention and treatment of depression as well as to promote psychological well-being in this at-risk group.

The main finding from the current research suggests that risk for depression in late chronotypes is related to the negative cognitive biases they display. One implication of this finding is to examine the effectiveness of early cognitive interventions in preventing the onset of depression in this population. For example, ‘cognitive bias modification’ (CBM) is a prevention technique that aims to modify maladaptive cognitive biases by training participants to process emotional information in a more positive/benign way. Participants are trained on different levels (e.g. attention, interpretation, memory) using well-validated tasks (e.g. dot-probe task) until an adaptive processing style is adopted and maintained (Koster & Hoorelbeke, 2015). Although the majority of CBM research to date has been applied to anxiety, some studies have shown that the technique is effective in reducing negative biases and symptoms in depression. For example, Yang and colleagues (2015) reported that participants with high levels of depressive symptoms (but were not clinically diagnosed with depression) showed a significant effect of attentional bias modification on reducing depressive symptoms compared to placebo which was maintained at three month follow-up (Yang, Ding, Dai, Peng, & Zhang, 2015). Similarly, Pictet and colleagues (2016) reported that imagery CBM (listening to positive scenarios and imagining them) had a significant effect of improving depressive symptoms, anhedonia and interpretation bias in a sample of participants with sub-threshold depressive symptoms (Pictet, Jermann, & Ceschi, 2016). In a meta-analysis of 45 CBM studies, Hallion

and colleagues (2011) reported that CBM exerted a small positive effect on anxious and depressive symptoms but this effect was only reliable in studies where participants also experienced a stressor, and was only found to significantly modify anxiety (Hallion & Ruscio, 2011). This suggests that CBM may only have a modest effect on improving depressive symptoms however, the authors also noted that only a small number of studies assessed measures of depression and many tasks that have been studied are specific to anxiety (e.g. attention bias to threat). A more recent meta-analysis of CBM studies identified a small significant effect on depression but also indicated problems of small sample sizes, poor quality trials and a high level of heterogeneity (Cristea, Kok, & Cuijpers, 2015). Therefore, larger studies with better randomisation protocols and follow up measures tailored specifically to depression are needed. Nonetheless, future research exploring whether CBM can be used to prevent depression onset in high risk populations including late chronotype individuals are warranted.

More generally, another implication of the present research relates to the idea of social jet lag: a mismatch between the individual's internal body clock and external demands. Merikanto and colleagues (2015) suggested that it may be beneficial for late chronotypes to adapt their daily activity schedule to match their delayed circadian clock thereby reducing the clash between their biological rhythm and external demands (Merikanto et al., 2015). Few experimental manipulations have investigated the impact of allowing individuals to follow, or better match, their circadian rhythm, however there is some evidence that it may be effective in reducing depressive symptomatology. For example, Vetter and colleagues (2015) reported increased well-being on weekdays when factory workers had their most strenuous shifts abolished (late evening shifts for early chronotypes and early morning shifts for late chronotype) (Vetter, Fischer, Matera, & Roenneberg, 2015). Boergers and colleagues (2014) reported that delaying school start time by 25 minutes was associated with a significant increase

in sleep duration and decrease in daytime sleepiness and depressive mood in school children (Boergers, Gable, & Owens, 2014). Similarly, another study reported significant improvements in depressive symptom scores and other health-related outcomes in response to a 30 minute delay in school start time (Owens, Belon, & Moss, 2010) and recently the American Academy of Sleep Medicine has called for American schools to implement start times of 08:30am or later based on increasing evidence for improved health and mood (Watson et al., 2017).

Alternatively, Skeldon and colleagues' mathematical model predicted that social jet lag would be more effectively reduced by decreasing the amount of evening light exposure compared to delaying work schedules (Skeldon, Phillips, & Dijk, 2017). The authors proposed that the effect of later wake-up times has only a transitory effect on social jet-lag and has a minimal effect on the more extreme late chronotypes who are most at risk; therefore reducing evening light exposure may be a more effective option (and arguably easier to implement). As noted above, night-time light exposure is thought to be a possible mechanism underlying the relationship between late chronotype and depression partly independent of social jet lag. Therefore, Muller and Haag (2017) suggested that abstaining from bright artificial light at night (e.g. computer, television and mobile phone screens) is an important preventative measure for depression in relation to late chronotype as well as improving exposure to natural day light (e.g. spending more time outdoors or sitting next to a window). Moreover, interventions to improve sleep quality such as employing good sleep hygiene and reducing caffeine and alcohol consumption before bed may also help to reduce circadian misalignment. Reducing the effects of social jet-lag, artificial light and poor sleep quality may have specific implications for the prevention of depression in adolescents as this population is thought to experience a greater degree of circadian misalignment. Interestingly, one study also indicated that 31-40 year olds experience higher levels of social jet lag and depressive symptoms due to 'the rush hour of life'

(Levandovski et al., 2011) suggesting strategies for reducing social jet lag and preventing depression may also be particularly relevant for this age group.

Finally, a large body of research has shown the efficacy of bright light therapy in treating Seasonal Affective Disorder (SAD) and non-seasonal depression with estimated effect sizes of 0.84 and 0.53 respectively (Golden et al., 2005). This therapy is thought to alleviate depressive symptoms by enhancing serotonergic activity in the brain and suppressing daytime melatonin levels causing a phase advance (earlier sleep timing) when applied in the morning (Oldham & Ciraulo, 2014). Additionally, administration of melatonin and melatonin agonists (such as agomelatine and sertraline) in the evening have been shown to cause phase advances and significant antidepressant effects (Kasper et al., 2010). Therefore, correcting phase disturbances with bright morning light (or reducing evening light) and melatonin administration may be useful strategies to reduce social jet-lag and improve mood in late chronotype individuals.

## 9.6. Future research

The chronotype literature surrounding risk for depression is sparse and the present research has highlighted a number of mechanisms that contribute to our understanding of this association. This research has also sparked a number of avenues warranting future investigation which are proposed here.

Firstly, there is still a need for large, well powered longitudinal studies. Such studies are needed to investigate the predictive power of negative cognitive biases, impaired reward sensitivity and emotion regulation (as identified here) for the development of depression. Indeed, only a certain number of late chronotypes will develop depression and so identifying these individuals will be the first step towards developing prevention strategies (e.g. CBM) for this risk group. As proposed by the diathesis-stress model, depression is caused by a

vulnerability combined with experience of stressful life events. It is therefore predicted that late chronotype represents a vulnerability trait for depression but that a trigger (e.g. a major life event) may be needed for the onset of depression. However, previous studies have not yet investigated this or how these factors might interact to cause depression. For example, several models of neuroticism have shown that exposure to stress (e.g. divorce, assault, job loss) directly increases risk for depression in highly neurotic individuals as they are more sensitive to the depressogenic effects of adversity than low risk individuals (Kendler et al., 2004). Therefore future research should focus on whether negative cognitive biases might predict depressive onset in late chronotypes and how this mechanism might interact with other factors such as stress. There is evidence to suggest that the timing of light exposure may underlie the relationship between late chronotype and depression and so the timing of stress exposure may also be important in the aetiology of depression in this group.

The resting-state findings reported in the present research suggest that altered connectivity within the default mode network might represent self-critical thoughts or impaired allocation of attentional resources. The behavioural data provided indirect evidence of self-critical thoughts (increased recognition and recall of negative self-referential personality trait words compared to positive words) and no evidence of attentional biases. However, no control condition was included in the self-referent paradigms and the dot-probe task used may not have captured disengagement of attention specifically. Therefore future research may seek to investigate differences in self-critical thoughts and attentional switching associated with chronotype using self-report, behavioural measures and fMRI. Similarly, other behavioural observations such as self-referent recognition and recall of words in relation to late chronotype would benefit from MRI studies that investigate how these biases are functionally determined in the brain.

Late chronotype was related to reduced reward sensitivity in this investigation, however this was inferred from a behavioural measure of risk taking. Therefore, tasks that probe reward (and punishment) sensitivity specifically are needed to replicate and further characterise this observation. These measures would be enriched by examining if they relate to self-report measures of anhedonia; symptoms associated with a blunted response to reward. Unexpectedly, late chronotypes recalled significantly fewer words overall than early chronotypes so future studies probing global memory and executive function would be useful to determine if any impairments relevant to depression exist in this risk group. Further characterisation of the cognitive mechanisms associated with late chronotype could be done by investigating differences in reward circuitry and prefrontal cortex activity using fMRI. Similarly, the current data suggest that later chronotypes display alterations in emotion processing circuitry, in particular; enhanced amygdala reactivity to negative stimuli and reduced connectivity with anterior cingulate responsible for emotion regulation. Therefore, future fMRI studies using emotion regulation tasks could be used to probe this association directly. Moreover, it is unclear why later chronotypes have altered connectivity but it appears to be unrelated to global differences in GABA and glutamate levels at rest. Future studies probing neurotransmitter differences in specific brain regions associated with emotion regulation e.g. ACC, DLPFC could be used as an indicator of impaired neurotransmitter function and could be integrated with the fMRI data in order to better understand emotion processing in relation to chronotype.

Finally, there is converging evidence for associations between late chronotype, increased social jet lag, poor sleep quality and increased light exposure at night, and these factors are also associated with depression. However, it is still unclear how these factors interact and the causal nature of these factors in the aetiology of depression. Although it is difficult to study these factors independently, future studies that aim to experimentally manipulate (reduce)

light exposure and social jet lag would allow us to understand the individual contributions better and help to inform strategies to promote well-being in late chronotypes.

## 9.7. Conclusions

In conclusion, the present work has provided evidence that negative biases in information processing exist in healthy, late chronotype individuals that are at increased risk for developing depression. The neural basis of altered facial expression processing was further demonstrated using fMRI and resting-state connectivity was shown to be altered in a key brain network. These behavioural and neural mechanisms may underlie risk for depression in late chronotypes and represent trait markers for the disorder. In contrast, structural differences and global alterations in neurometabolites were not related to risk for depression and may only exist during the depressed state. The present set of experiments has generated novel findings using a range of behavioural tasks and neuroimaging techniques and has important implications for the treatment and prevention of depression. It has also paved the way for future studies to investigate the predictive power of these findings and to evaluate the efficacy of interventions that promote psychological well-being in this population.



## 10. References

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## **11. Appendices**

Appendices include consent forms, debrief forms and questionnaires/scales used across all three studies. Materials are presented for each study in order: behavioural study (Chapter 2), online study (Chapter 3) and neuroimaging study (Chapters 5 – 8).

**Title of Research Project: Investigating the interactions between chronotype and emotional and cognitive processing.**

Ethics reference N<sup>o</sup>: PSYCH 15/197

Participant N<sup>o</sup>:

### **Background**

This study aims to investigate the interactions between chronotype (i.e. whether you are a night owl or a morning lark) and emotional and cognitive processing. The study will include up to fifty participants and will take place at the University of Roehampton.

### **What the research involves**

We will first ask you to provide some basic background information (e.g. age, gender, approximately how many cigarettes you smoke per day (if any) and how much alcohol (if any) you consume) and complete three questionnaires assessing mood, anxiety and risk taking. Some of these questions are of a sensitive nature. If you do not wish to or are uncomfortable answering any of the questions presented please leave them blank. Immediately following the questionnaires you will be invited to complete some simple tasks on a computer investigating memory, emotional processing and risk taking. It is anticipated that your participation should take no longer than 90 minutes and you will be rewarded 1.5 SONA credits for your participation.

If you wish to withdraw from the study at any point, you are free to do so without justification. You may also withdraw your data once the study is complete. For your data to be withdrawn after completion, please contact the investigator and quote your participant number. This is recorded on your debrief form. If you are a student completing this study in order to gain credits, please be aware that withdrawing from the study will not affect this.

Please return the consent form to the investigator and retain the debrief form. All data gathered during this study will be anonymised and stored securely. Signed consent forms and completed questionnaires will be stored separately in locked filing cabinets. It will not be possible to link questionnaires to consent forms. Only researchers directly involved in this study will have access to your data.

### **Principal Investigator Contact Details:**

Charlotte Horne  
Department of Psychology  
Roehampton University  
Whitelands College  
Holybourne Avenue  
London SW15 4JD

Email: [hornecl@roehampton.ac.uk](mailto:hornecl@roehampton.ac.uk)

### Consent Statement:

I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator within the limits described and that my identity will be protected in the publication of any findings and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.

My email address is \_\_\_\_\_@roehampton.ac.uk

Participant name (Please print clearly) .....

Signature .....

Date .....

We may wish to contact you by email about future studies. In particular we will want to include participants from the full range of chronotypes (early birds right through to night owls). If you think you would like to take part in further research related to this topic please indicate this below and enter your email address in the space provided. If you leave this section blank we will assume that you do not wish to be contacted in the future. Please note, you are under no obligation to take part in future studies.

Yes I would like to be contacted in the future ☐

Please note: if you have a concern about any aspect of your participation or any other queries please raise these with the investigator or project supervisor (or if the researcher is a student then you can also contact the Director of Studies). However if you would like to contact an independent party please contact the Head of Department:

**Project Supervisor:**

Dr Ray Norbury  
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**Head of Psychology:**

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Holybourne Avenue  
London SW15 4JD  
[d.bray@roehampton.ac.uk](mailto:d.bray@roehampton.ac.uk)  
Tel: 020 8392 3627

*Participant Number:*

*Unique study code: PSYCH 15/197*

## **PARTICIPANT DEBRIEF**

### **Title of Research Project: Investigating the association between chronotype and emotional processing biases**

Thank you for taking part in this study, we greatly appreciate your contribution.

There is evidence to suggest that late chronotype impacts on cognition. We do not know, however, if emotional processing is also altered. The aim of this study was to investigate how chronotype interacts with emotional processing. During this session you were given a surprise memory test. We did not alert you to this component of the session as we wanted you to focus on the likeability/dislikeability of the words (the first task you completed).

All data gathered during this study will be held securely. If you wish to withdraw from the study please contact the PI with your participant number (above), and your data will be removed.

Please note: if you have a concern about any aspect of your participation or any other queries please raise these with the investigator or project supervisor (or if the researcher is a student then you can also contact the Director of Studies). However if you would like to contact an independent party please contact the Head of Department:

#### **Investigator**

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Tel: 020 8392 3744

If you are a student at Roehampton University and are troubled or worried about any aspect of the study, or issues it may have raised, you may find it helpful to contact one of the following who will be able to advise you on agencies that can deal with your particular concern:

**Student Welfare Officer:** [louise.walton@roehampton.ac.uk](mailto:louise.walton@roehampton.ac.uk)

Student Medical Centre  
Old Court  
Froebel College  
Roehampton Lane  
London SW15 5PJ

Tel: 020 8392 3679  
waccg.[studentmedicalcentre@nhs.net](mailto:studentmedicalcentre@nhs.net)

**CASE REPORT FORM**

**Investigating the association between chronotype and emotional processing biases.**

Study reference number

**SITE:**

**Psychology Department, University of  
Roehampton**

Participant number:

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*I am confident that the information supplied in this case record form is complete and accurate data. I confirm that the study was conducted in accordance with the protocol and any protocol amendments and that written informed consent was obtained prior to the study.*

Investigator's Signature: .....

Date of signature:

D	D	M	M	M	Y	Y	Y	Y

## **Testing Day**

**Date:** \_\_\_\_\_  
DD MM YYYY

### **Inclusion Criteria**

**Yes**

**No\***

**1** Willing and able to provide informed consent

☐☐

### **DEMOGRAPHIC DATA**

#### **D.O.B:**

Age (yrs):

Sex:

Female

☐

Male

☐

Number of cigarettes smoked per day:

Units of alcohol consumed per week:

Do you know of any family member that is currently depressed or has been diagnosed as depressed in the past? Mother /Father/Grandparents

Have you in the past or are currently diagnosed with depression? Y/N

Have you been diagnosed with any sort of sleeping disorder? Y/N

### **Additional comments/notes**



## Beck Depression Inventory

Please read each item carefully and circle the number next to the answer that best describes how you have been feeling the past week.

1.     0     I do not feel sad  
       1     I feel sad  
       2     I am sad all the time and can't snap out of it  
       3     I am so sad or unhappy that I can't stand it
  
2.     0     I am not particularly discouraged about the future  
       1     I feel discouraged about the future  
       2     I feel I have nothing to look forward to  
       3     I feel that the future is hopeless and that things cannot improve
  
3.     0     I do not feel like a failure  
       1     I feel I have failed more than the average person  
       2     As I look back on my life, all I can see is a lot of failures  
       3     I feel I am a complete failure as a person
  
4.     0     I get as much satisfaction out of things as I used to  
       1     I don't enjoy things the way I used to  
       2     I don't get real satisfaction out of anything anymore  
       3     I am dissatisfied or bored with everything
  
5.     0     I don't feel particularly guilty  
       1     I feel guilty a good part of the time  
       2     I feel quite guilty most of the time  
       3     I feel guilty all of the time
  
6.     0     I don't feel I am being punished  
       1     I feel I may be punished  
       2     I expect to be punished  
       3     I feel I am being punished
  
7.     0     I don't feel disappointed in myself  
       1     I am disappointed in myself  
       2     I am disgusted with myself  
       3     I hate myself
  
8.     0     I don't feel I am any worse than anybody else  
       1     I am critical of myself for my weaknesses or mistakes  
       2     I blame myself all the time for my faults  
       3     I blame myself for everything bad that happens
  
9.     0     I don't have any thoughts of killing myself  
       1     I have thoughts of killing myself, but I would not carry them out  
       2     I would like to kill myself

- 3 I would kill myself if I had the chance
10. 0 I don't cry any more than usual  
1 I cry more now than I used to  
2 I cry all the time now  
3 I used to be able to cry, but now I can't cry even though I want to
11. 0. I am no more irritated now than I ever am  
1 I get annoyed or irritated more easily than I used to  
2 I feel irritated all the time now  
3 I don't get irritated at all by the things that used to irritate me
12. 0 I have not lost interest in other people  
1 I am less interested in other people than I used to be  
2 I have lost most of my interest in other people  
3 I have lost all of my interest in other people
13. 0 I make decisions about as well as I ever could  
1 I put off making decisions more than I used to  
2 I have greater difficulty in making decisions than before  
3 I can't make decisions at all anymore
14. 0 I don't feel I look any worse than I used to  
1 I am worried that I am looking old and unattractive  
2 I feel that there are permanent changes in my appearance that make me look unattractive  
3 I believe that I look ugly
15. 0 I can work about as well as before  
1 It takes an extra effort to get started at doing something  
2 I have to push myself very hard to do anything  
3 I can't do any work at all
16. 0 I can sleep as well as usual  
1 I don't sleep as well as I used to  
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep  
3 I wake up several hours earlier than I used to and cannot get back to sleep
17. 0 I don't get more tired than usual  
1 I get tired more easily than I used to  
2 I get tired from doing almost anything  
3 I am tired too tired to do anything
18. 0 My appetite is no worse than usual  
1 My appetite is not as good as it used to be  
2 My appetite is much worse now  
3 I have no appetite at all anymore

19. 0 I haven't lost much weight, if any, lately  
1 I have lost more than 5 pounds  
2 I have lost more than 10 pounds  
3 I have lost more than 15 pounds
20. 0 I am no more worried about my health than usual  
1 I am worried about physical problems such as aches and pains; or  
upset stomach; or constipation  
2 I am very worried about physical problems and it is hard to think about much else  
3 I am so worried about my physical problems that I cannot think about anything  
else
21. 0 I have not noticed any recent changes in my interest in sex  
1 I am less interested in sex than I used to be  
2 I am much less interested in sex now  
3 I have lost interest in sex completely

## **STATE QUESTIONNAIRE**

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right now*, that is, *at this moment in time*. There are no right and wrong answers. Do not spend too much time on each statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately	Very much
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

## **TRAIT QUESTIONNAIRE**

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you **GENERALLY** feel. There are no right and wrong answers. Do not spend too much time on each statement but give the answer which seems to describe you best.

	<b>Not at all</b>	<b>Somewhat</b>	<b>Moderately</b>	<b>Very much</b>
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am 'cool, calm and collected'	1	2	3	4
8. I feel that the difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that doesn't really matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thoughts run through my mind and bother me	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over recent concerns and interests	1	2	3	4

## MORNINGNESS-EVENINGNESS QUESTIONNAIRE

For each question, please select the answer that best describes you by circling the point value that best indicates how you have felt in recent weeks.

1. *Approximately* what time would you get up if you were entirely free to plan your day?

[5] 5:00 AM–6:30 AM (05:00–06:30 h)

[4] 6:30 AM–7:45 AM (06:30–07:45 h)

[3] 7:45 AM–9:45 AM (07:45–09:45 h)

[2] 9:45 AM–11:00 AM (09:45–11:00 h)

[1] 11:00 AM–12 noon (11:00–12:00 h)

2. *Approximately* what time would you go to bed if you were entirely free to plan your evening?

[5] 8:00 PM–9:00 PM (20:00–21:00 h)

[4] 9:00 PM–10:15 PM (21:00–22:15 h)

[3] 10:15 PM–12:30 AM (22:15–00:30 h)

[2] 12:30 AM–1:45 AM (00:30–01:45 h)

[1] 1:45 AM–3:00 AM (01:45–03:00 h)

3. If you usually have to get up at a specific time in the morning, how much do you depend on an alarm clock?

[4] Not at all

[3] Slightly

[2] Somewhat

[1] Very much

4. How easy do you find it to get up in the morning (when you are not awakened unexpectedly)?

[1] Very difficult

[2] Somewhat difficult

[3] Fairly easy

[4] Very easy

5. How alert do you feel during the first half hour after you wake up in the morning?

[1] Not at all alert

[2] Slightly alert

[3] Fairly alert

[4] Very alert

6. How hungry do you feel during the first half hour after you wake up?

[1] Not at all hungry

[2] Slightly hungry

[3] Fairly hungry

[4] Very hungry

7. During the first half hour after you wake up in the morning, how do you feel?

[1] Very tired

[2] Fairly tired

[3] Fairly refreshed

[4] Very refreshed

8. If you had no commitments the next day, what time would you go to bed compared to your usual bedtime?

[4] Seldom or never later

[3] Less than 1 hour later

[2] 1-2 hours later

[1] More than 2 hours later

9. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week, and the best time for him is between 7-8 AM (07-08 h). Bearing in mind nothing but your own internal "clock," how do you think you would perform?

[4] Would be in good form

[3] Would be in reasonable form

[2] Would find it difficult

[1] Would find it very difficult

10. At *approximately* what time in the evening do you feel tired, and, as a result, in need of sleep?

[5] 8:00 PM–9:00 PM (20:00–21:00 h)

[4] 9:00 PM–10:15 PM (21:00–22:15 h)

[3] 10:15 PM–12:45 AM (22:15–00:45 h)

[2] 12:45 AM–2:00 AM (00:45–02:00 h)

[1] 2:00 AM–3:00 AM (02:00–03:00 h)

11. You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last two hours. You are entirely free to plan your day. Considering only your "internal clock," which one of the four testing times would you choose?

[6] 8 AM–10 AM (08–10 h)

[4] 11 AM–1 PM (11–13 h)

[2] 3 PM–5 PM (15–17 h)

[0] 7 PM–9 PM (19–21 h)

12. If you got into bed at 11 PM (23 h), how tired would you be?

[0] Not at all tired

[2] A little tired

[3] Fairly tired

[5] Very tired

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which one of the following are you most likely to do?

[4] Will wake up at usual time, but will not fall back asleep

[3] Will wake up at usual time and will doze thereafter

[2] Will wake up at usual time, but will fall asleep again

[1] Will not wake up until later than usual

14. One night you have to remain awake between 4-6 AM (04-06 h) in order to carry out a night watch. You have no time commitments the next day. Which one of the alternatives would suit you best?

- [1] Would not go to bed until the watch is over
- [2] Would take a nap before and sleep after
- [3] Would take a good sleep before and nap after
- [4] Would sleep only before the watch

15. You have two hours of hard physical work. You are entirely free to plan your day. Considering only your internal “clock,” which of the following times would you choose?

- [4] 8 AM–10 AM (08–10 h)
- [3] 11 AM–1 PM (11–13 h)
- [2] 3 PM–5 PM (15–17 h)
- [1] 7 PM–9 PM (19–21 h)

16. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week. The best time for her is between 10-11 PM (22-23 h). Bearing in mind only your internal “clock,” how well do you think you would perform?

- [1] Would be in good form
- [2] Would be in reasonable form
- [3] Would find it difficult
- [4] Would find it very difficult

17. Suppose you can choose your own work hours. Assume that you work a five-hour day (including breaks), your job is interesting, and you are paid based on your performance. At *approximately* what time would you choose to begin?

- [5] 5 hours starting between 4–8 AM (05–08 h)
- [4] 5 hours starting between 8–9 AM (08–09 h)
- [3] 5 hours starting between 9 AM–2 PM (09–14 h)
- [2] 5 hours starting between 2–5 PM (14–17 h)
- [1] 5 hours starting between 5 PM–4 AM (17–04 h)

18. At *approximately* what time of day do you usually feel your best?

- [5] 5–8 AM (05–08 h)
- [4] 8–10 AM (08–10 h)
- [3] 10 AM–5 PM (10–17 h)
- [2] 5–10 PM (17–22 h)
- [1] 10 PM–5 AM (22–05 h)

19. One hears about “morning types” and “evening types”. Which one of these types do you consider yourself to be?

- [6] Definitely a morning type
- [4] Rather more a morning than an evening type
- [2] Rather more an evening than a morning type
- [1] Definitely an evening type



# The Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions. During the past month,

1. When have you usually gone to bed? \_\_\_\_\_
2. How long (in minutes) has it taken you to fall asleep each night? \_\_\_\_\_
3. When have you usually gotten up in the morning? \_\_\_\_\_
4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) \_\_\_\_\_

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)
9. During the past month, how would you rate your sleep quality overall?				

- Component 1 #9 Score..... C1 \_\_\_\_\_
- Component 2 #2 Score ( $\leq 15$  min=0; 16-30 min=1; 31-60 min=2, >60 min=3) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3) ..... C2 \_\_\_\_\_
- Component 3 #4 Score ( $> 7=0$ ; 6-7=1; 5-6=2;  $< 5=3$ ) ..... C3 \_\_\_\_\_
- Component 4 (total # of hours asleep)/(total # of hours in bed) x 100  
 $> 85\%=0$ ,  $75\%-84\%=1$ ,  $65\%-74\%=2$ ,  $< 65\%=3$  ..... C4 \_\_\_\_\_
- Component 5 Sum of Scores #5b to #5j (0=0; 1-9=1; 10-18=2; 19-27=3)..... C5 \_\_\_\_\_
- Component 6 #6 Score ..... C6 \_\_\_\_\_
- Component 7 #7 Score + #8 Score (0=0; 1-2=1; 3-4=2; 5-6=3) ..... C7 \_\_\_\_\_

Add the seven component scores together \_\_\_\_\_ **Global PSQI Score** \_\_\_\_\_

**EYSENCK PERSONALITY QUESTIONNAIRE-REVISED**

Answer each of the following questions for who you are now. There are no right or wrong answers, and no trick questions. Work quickly and do not think too long about the exact meaning of the questions.

1	Does your mood often go up and down?	Yes	No
2	Do you ever feel 'just miserable' for no reason?	Yes	No
3	Do you often worry about things you should not have done or said?	Yes	No
4	Are you an irritable person?	Yes	No
5	Are your feelings easily hurt?	Yes	No
6	Do you often feel 'fed-up'?	Yes	No
7	Are you often troubled about feelings of guilt?	Yes	No
8	Would you call yourself a nervous person?	Yes	No
9	Are you a worrier?	Yes	No
10	Do you worry about awful things that might happen?	Yes	No
11	Would you call yourself tense or 'highly strung'?	Yes	No
12	Do you worry about your health?	Yes	No
13	Do you suffer from sleeplessness?	Yes	No
14	Have you often felt listless and tired for no reason?	Yes	No
15	Do you often feel life is very dull?	Yes	No
16	Do you worry a lot about your looks?	Yes	No
17	Have you ever wished that you were dead?	Yes	No
18	Do you worry too long after an embarrassing experience?	Yes	No
19	Do you suffer from 'nerves'?	Yes	No
20	Do you often feel lonely?	Yes	No
21	Are you easily hurt when people find fault with you or the work you do?	Yes	No
22	Are you sometimes bubbling over with energy and sometimes very sluggish?	Yes	No
23	Are you touchy about some things?	Yes	No
24	When your temper rises, do you find it difficult to control?	Yes	No

## **INSTRUCTIONS FOR THE COMPUTER TASKS**

1. Encoding. E-Prime folder 1\_UR\_Encoding. Start E-Prime the UR\_Encoding task and read the following instructions to the participant:

***“ We are now going to show you a series of words. For each word imagine you overheard someone describe you using this word and indicate if you would like or dislike to be described in this way. For example, if you overheard someone describe you as annoying, would you be pleased or displeased? Please use the keyboard to indicate your response by pressing L for Like or D for Dislike. If you are unsure of the meaning of the word take a guess. Please place the index finger of your left hand on the letter D and the index finger of your right hand on the letter L on the keyboard and we will begin. ”***

2. Expression recognition. E-Prime folder 2\_UR\_Expression. Start the E-Prime UR\_Expression task and read the following instructions to the participant:

***“ We are now going to show you a series of faces. Use the mouse to indicate if the expression displayed on the screen is Happy, Neutral or Sad. Please respond quickly and accurately. Use your ‘gut’ feeling in you are unsure what the emotion is. If you think you have made a mistake do not worry, just wait for the next trial to start. We will start with a few examples.***

3. Balloon Analogue Risk Task. E-Prime folder 3\_UR\_bart. Start the E-Prime UR\_BART\_BEHAVIOUR task and read the following instructions to the participant:

***“The aim of this task is to win as many points as possible. On each trial you will see a digital balloon. You can choose to “pump up” the balloon by pressing the left arrow key or “cash out” by pressing the right arrow key. If you choose to pump the balloon (i.e. press the left arrow) the balloon will expand and the value of the balloon will increase by 5 points. If you choose to cash out (i.e. press the right arrow) the points for the current trial will be added to your banked points. Remember each time you pump you take a risk of the balloon exploding and losing the points earned on the current trial (previously banked points will not be affected). There are a set number of trials in this experiment, so try to maximise your points on each trial.”***

4. Recall/Recognition. E-Prime folder 4\_UR\_Recognition. Start the E-Prime UR\_Recognition task and read the following instructions to the participant:

***“ We are now going to test your memory for the words shown in the first experiment (when you had to judge them as likable or dislikeable). First, please just type as many words as you can recall from memory, if you make a mistake use the backspace and please hit the spacebar between words. Once you have typed as many words as you can remember please hit the enter/return key. We will then show you a series of words. Please use the keyboard to indicate whether the word on the screen is OLD (i.e. one that you have seen during the first experiment) or NEW (i.e. a word that you have not seen***

*during the first experiment). Press O for old, N for new. In this experiment do not worry about whether you “Like” or “Dislike” the word, that is not important.*

5. Dot Probe task. E-Prime folder 5\_UR\_Dot\_Probe. Start the E-Prime UR\_Dot\_Probe task and read the following instructions to the participant:

*“We are now going to show you two faces on the screen at a time. They will be immediately followed by an asterix on either the left or right side of the computer screen. Please indicate with the “z” and “m” keys on the keyboard which side the asterix appeared on (if on the left press z, if on the right press m). Please respond as quickly and as accurately as you can. If you think you have made a mistake do not worry, just wait for the next trail to appear. Please place the index finger of your left hand on the letter z and the index finger of your right hand on the letter m on the keyboard and we will begin. ”*

## Consent and Information form for Online Study: “Exploring emotional processing in 'larks' and 'owls'”.

Thank you for your interest in my study. Before you decide to take part, it is important for you to understand the nature of the study. Please take the time to carefully read the following details. You are welcome to discuss it with others. If there is anything that is unclear, or if you would like more information, please contact [hornec1@roehampton.ac.uk](mailto:hornec1@roehampton.ac.uk).

This study aims to explore the interactions between daytime preferences (do you like to wake early and go to bed early or do you prefer to sleep late and go to bed late) and emotional processing. During this questionnaire package you will be invited to complete a number of questionnaires that assess your sleep patterns and quality of sleep. We will then show a series of images and ask you to indicate the expression displayed on the face (sad, happy or neutral).

All data relating to your completion of this online questionnaire will be stored and processed on password protected computers and will be available only to researchers directly involved in the study or approved members of the University of Roehampton. You are advised not to provide your name in order to ensure anonymity and confidentiality. Your identity will not be passed on to unauthorised persons and will be protected in the publication of any findings.

You are under no obligation to complete the online questionnaire, you can also request for your data to be withdrawn at any time once the questionnaire has been submitted, by emailing the Principal Investigator ([hornec1@roehampton.ac.uk](mailto:hornec1@roehampton.ac.uk)) with your self-generated identifier.

This questionnaire package should take around 25-30 minutes to complete. You cannot save your progress, so please ensure that you complete the questionnaire in one sitting. If you backspace or let your browser time out your data will be lost.

Thank you!

Agree	Disagree
<input type="radio"/>	



**Title of Research Project:** Happy as a lark: Study 2

**Brief Description of Research Project, and What Participation Involves:**

You are being invited to take part in a research project. Before you decide if you want to participate, it is important for you to fully understand why the research is being done and what it will involve. Please take time to read this consent form carefully. If there is anything you do not understand, or if you would like more information, please feel free to ask any questions.

*What is the purpose of the study?*

We are interested in how the brain responds to different types of information (e.g. taking a risky decision or looking at an emotional face) and how this may differ depending on whether you are an early chronotype (i.e. someone who likes to go to bed early and wake up early) or a late chronotype (i.e. someone who prefers to go to bed late and sleep late in the morning). To do this we will use Functional Magnetic Resonance Imaging (fMRI) and Magnetic Resonance Spectroscopy (MRS) to explore which regions of the brain are utilised when we process these types of information. We hope that the results of this study may improve our understanding of late and early chronotype and how the brain processes emotional information and risky choices.

*Do I have to take part?*

No. It is up to you to decide whether or not to take part and you are free to withdraw at any time without giving a reason. There is no compulsion or academic pressure to take part in this project and if you decline to participate or subsequently withdraw your course marks or any other academic activity will not be adversely affected.

*Why have I been chosen?*

In this study we are interested in studying up to 40 right-handed healthy people, between the ages of 18 and 60. You have been selected to participate in this study because you have participated in a previous study measuring chronotype (whether you are a 'morning lark' or 'night owl') and emotional processing. From completing the questionnaires in the previous studies, you have been identified as either a definite-morning or definite-evening chronotype which is of particular interest for this study.

To ensure that it is safe for you take part in this study you must answer to NO to the following 15 questions. If you answer yes to any these you will **NOT** be able to take part in this study. In addition you will not be able to take part in the study if you are claustrophobic.

1. Have you been fitted with a pacemaker or artificial heart valve?
2. Have you any aneurysm clips or shunts in your body, or a cochlear implant?
3. Have you ever had any metal fragments in your eyes?

4. Have you ever had any metal fragments, e.g. shrapnel in any other part of your body?
5. Have you any surgically implanted metal in any part of your body, other than dental fillings and crowns (e.g. joint replacement or bone reconstruction)
6. Have you ever had any surgery that might have involved metal implants of which you are not aware?
7. Do you wear a denture plate or brace with metal in it?
8. Do you wear a hearing aid?
9. Do you use drug patches attached to your skin?
10. Have you ever suffered from any of: epilepsy, diabetes or thermoregulatory problems?
11. Have you ever suffered from any heart disease?
12. Is there any possibility that you might be pregnant?
13. Have you been sterilised using clips?
14. Do you have a contraceptive coil (IUD) installed?
15. Are you currently breast-feeding an infant?

What will the study involve?

The study will involve a single session outlined below:

We will ask you to:

- Come to the Combined Universities Brain Imaging Centre (CUBIC), which is located at Royal Holloway College, Egham
- Complete a standardised MRI screening
- During the Magnetic Resonance Imaging (MRI) session we will acquire a structural scan of your brain and also carry out a number of functional scans during rest and while making risky decisions and while viewing faces depicting happy and fearful facial expressions. During the resting scan all you need to do is watch a cross on the screen and let your mind wander (i.e. do not focus on anything in particular). During the risky decision task you will be asked to press one of two buttons to either inflate (pump) a computer-simulated balloon image or to “cash out”. Each trial will begin with the presentation of a balloon and end when the balloon explodes or you cash out. Each pump will increase the potential number of points on a given trial, and the number of points accumulated in a temporary bank. You can cash out and keep the points accumulated at any point during the trial. If a balloon explodes, the trial will provide no points, but points from previous trials would be unaffected. The aim of the game is to maximise the number points earned. The task will be presented on a personal computer and rendered viewable to you by an angled mirror located in the scanner bore. Before starting the nature of the task will be explained to you and you will be given the opportunity to complete a few practice trials to ensure you fully understand the task. During the faces task we will show you pictures of happy and fearful faces and ask you to indicate by button press whether the face on the screen is male or female. Just as for the risk decision task the faces will be presented on a personal computer and rendered viewable to you by an angled mirror located in the scanner bore. During the emotional stroop task we will show you a series of words on the screen and you simply indicate, by button press, the number of words displayed. Before we start each task we will make sure you are happy with the instructions and comfortable with the task. The functional tasks should not take more than 40 minutes to complete.

- Once the functional tasks are completed we will carry out the Magnetic Resonance Spectroscopy (MRS) scans. During the MRS scans all we ask you to do is relax and keep as still as possible. We will collect a single MRS scan lasting approximately 10 minutes in duration.
- The total time you will be in the scanner should not be more than approximately 75 minutes.
- Once the scan is finished you will be escorted back to the control room and invited to complete 4 questionnaires measuring your mood, anxiety levels, your chronotype and sleep quality. The total time to complete the questionnaires should not be more than 20 minutes.
- We anticipate that the total time you will spend at CUBIC will be approximately 2 hours.

### What is an FMRI scan?

Functional MRI is a totally safe, non-invasive procedure that uses strong magnetic fields to look at your brain (this type of scan does not involve the use of any ionising radiation [x-rays]). During the scan you will be lying inside a long, quite narrow tube (so it is important that you are not claustrophobic). Reflecting mirrors, mounted on a plastic surround, are fitted in a position that allows you to view a screen placed at the back of the scanner. It is on this screen that we will present the risky decision task. The scans are quite noisy so we give you ear protection and we also give you an alarm call (a soft rubber bulb) you can squeeze at any time if you are feeling uncomfortable or want to be removed from the scanner. A fully trained CUBIC scan operator will go through the MRI screening form before you go into the scanner to make sure you are safe to enter the magnetic environment. The researchers and scan operator will be able to see you throughout the duration of the scan and will talk to you at regular intervals. Please, on the day of the scan wear comfortable clothes with minimal metal buttons or buckles.

### What are the risks and benefits?

All MRI procedures will be conducted in accordance with the rigorous safety procedures in place at CUBIC, and therefore do not pose any significant risk. Prior to scanning you will be asked to complete a standardised safety screening form to ensure you have no contra-indications for MR imaging. Some people may find the space limitation in the scanner unpleasant, but you will be given the opportunity to view the scanner before the study starts. The scans are quite noisy so we give you ear protection which you will need to wear throughout the duration of the scan and we also give you an alarm button (a soft rubber bulb) you can squeeze at any time if you are feeling uncomfortable or want to be removed from the scanner.

CUBIC is wholly research orientated. As such, brain images acquired there are for specific research purposes only and are not suitable for diagnostic opinions. However, although not diagnostic scans, in the unlikely event of a possible structural abnormality being noted incidentally, we will contact your GP by letter. You will not be allowed to take part in the study unless you consent for us to contact your GP AND provide us with your current GP contact details.

Your help and the information we get from the study may improve our understanding of how chronotype impacts on brain structure and function. Eligible students will also receive 5 SONA credits.



### What if there is a problem?

You will be given the contact details of the lead investigator involved in the study (details are included in the Debrief form), and you will be able to contact them if you have any concerns during your participation in the study.

If you wish to complain about any aspect of the way you have been approached or treated during the course of this study you should contact Dr Ray Norbury. Alternatively, you may wish to contact Dr Diane Bray (Psychology, Head of Department). Contact details for Drs Ray Norbury and Diane Bray are included on the Debrief form.

### Will my taking part in the study be kept confidential?

In some instances we may be required to release your details to your GP (please see “*What are risks and benefits*” above). Otherwise, all the information about your participation in this study will be kept strictly confidential. Your results will be coded with a participant number and no personal information will be attached to the data. This anonymisation will occur at the earliest point of data collection. Data will be stored on a University computer for 10 years, while personal details will be stored separately in a locked filing cabinet. Only the named researchers and responsible individuals from the University of Roehampton will have access to this data. The overall results of the study may be published in scientific journals. However, all personal data will remain confidential, and no data relating to individual participants will be published.

Responsible members of the University of may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations. All will have a duty of confidentiality to you as a research participant.

What will happen to the results of the research study?

The results of this study may be published in scientific journals. However, no information which could be used to identify any individual participant will be published. If you are interested in finding out about the results of this research, please let us know, and we will make arrangements to inform you once the study is completed.

### **Investigator Contact Details:**

Charlotte Horne  
Department of Psychology  
Whitelands College  
University of Roehampton  
Holybourne Avenue  
London SW15 4JD  
E: hornec1@roehampton.ac.uk

### **Consent Statement:**

I agree to take part in this research, and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University’s Data Protection Policy.

Name .....

Signature .....

Date .....

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator (or if the researcher is a student you can also contact the Director of Studies.) However, if you would like to contact an independent party please contact the Head of Department.

**Supervisor**

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**Head of Psychology**

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## Reduced Morningness Eveningness Questionnaire (rMEQ)

**INSTRUCTIONS:** Please read each question carefully. Answer all questions as honestly as possible. Each question should be answered independently of others. Do NOT go back and check your answers.

1. Approximately what time would you get up if you were entirely free to plan your day?

5:00 - 6:30 am      6:30 - 7:45 am      7:45 - 9:45      9:45 - 11:00 am      11:am - 12 noon

☐☐☐☐☐

2. During the first half-hour after you wakeup in the morning, how tired do you feel?

Very tired

Fairly tired

Fairly refreshed

Very refreshed

☐☐☐☐

3. At approximately what time of the evening do you feel you become tired, and, as a result, in of need for sleep?

8:00 - 9:00 pm

9:00 - 10:15 pm

10:15 - 12:45 pm

12:45 pm - 2:00 am

2:00 - 3:00 am

☐☐☐☐☐

4. At approximately what time of the day do you think that you reach your “feeling best” peak?

5:00 - 8:00 am

8:00 - 10:00 am

10:00 am - 5:00 pm

5:00 - 10:00 pm

10:00 pm - 5:00 am

☐☐☐☐☐

5. One hears about “morning” and “evening” types of people. Which ONE of these types do you consider yourself to be?

Definitely morning

Rather more a morning

Rather more a evening

Definitely an evening

type

than an evening type

than a morning type

type

☐☐☐☐

## PHQ-4

Over the <u>last 2 weeks</u> , how often have you been bothered by the following problems? (Use “✓” to indicate your answer)	Not at all	Several days	More than half days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Little interest or pleasure in doing things	0	1	2	3
4. Feeling down, depressed, or hopeless	0	1	2	3

### Scoring

PHQ-4 total score ranges from 0 to 12, with categories of psychological distress being:

- None 0-2
- Mild 3-5
- Moderate 6-8
- Severe 9-12

Anxiety subscale = sum of items 1 and 2 (score range, 0 to 6)

Depression subscale = sum of items 3 and 4 (score range, 0 to 6)

On each subscale, a score of 3 or greater is considered positive for screening purposes

The PHQ scales were developed by Drs. Robert L. Spitzer, Janet B.W. Williams, and Kurt Kroenke and colleagues. The PHQ scales are free to use. For research information, contact Dr. Kroenke at [kkroenke@regenstrief.org](mailto:kkroenke@regenstrief.org)

Kroenke K, Spitzer RL, Williams JBW, Löwe B. An ultra-brief screening scale for anxiety and depression: the PHQ-4 Psychosomatics 2009;50:613-621.

Participant Number: N15\_01\_

## PARTICIPANT DEBRIEF

Reference N°:



**Title of Research Project:** Happy as a lark: Study 2

**Thank you very much for taking part in our study, we greatly appreciate your contribution.**

This study was designed to investigate how activity in specific areas of the brain (e.g. the dorsolateral prefrontal cortex and amygdala) that are typically activated by risky choices and emotional processing differs between late and early chronotype individuals. To do this we asked you to complete a series of simple tasks in the scanner and also complete some questionnaires after the scan.

In some instances we may be required to release your details to your GP (detailed in “*What are risks and benefits*” on the study consent form). Otherwise, all the information about your participation in this study will be kept strictly confidential. Your results will be coded with a participant number and no personal information will be attached to the data. This anonymisation will occur at the earliest point of data collection. Data will be stored on a University computer for 10 years, while personal details will be stored separately in a locked filing cabinet. Only the named researchers and responsible individuals from the University of Roehampton will have access to these data. The overall results of the study may be published in scientific journals. However, all personal data will remain confidential, and no data relating to individual participants will be published. Responsible members of the University of may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations. All will have a duty of confidentiality to you as a research participant.

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator (or if the researcher is a student you can also contact the Director of Studies.) However, if you would like to contact an independent party please contact the Head of Department.

**Investigator**

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If you are a student at Roehampton University and are troubled or worried about any aspect of the study, or issues it may have raised, you may find it helpful to contact the **Student Welfare Officer:** louise.walton@roehampton.ac.uk; Ext 3502. If you feel your concerns are more serious or complex you may wish to contact the **Student Medical Centre** on **Ext 3679**.

If you are a non-student your GP should be able to advise you on agencies that can deal with your particular concern.